

**EVALUATING EFFECTS OF NO-TILL COVER CROPPING SYSTEMS ON
INDIGENOUS ENTOMOPATHOGENIC NEMATODES AND FUNGI**

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ABSTRACT

Entomopathogens, entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF), are promising biocontrol agents for insect pest management. Use of entomopathens in Hawaii is challenged by quarantine restrictions and the failure of introduced entomopathens to persist in the field. This research focused on enhancing indigenous EPN (unknown *Heterorhabditis* sp.) populations in the field through conservation agriculture. We hypothesized that cover cropping with black oats (*Avena strigose*) or oil radish (*Raphanus sativus* ssp. *oleiferus*) followed by a no-till practice in an Oxisol soil would provide a favorable environment for EPN via provision of an organic mulch, reduced soil disturbance, improved water conservation, attracting alternative insect host and possibly production of herbivore-induced volatiles (HIPVs) from cover crops. Two field experiments, Oil Radish (OR) Experiment and Black Oat (BO) Experiment, both repeated once, were conducted to compare pre-plant treatments of 1) black oat (BO) or oil radish (OR) as cover crops in no-till plots, 2) bare ground (BG) followed by conventional tillage, and 3) conventional tillage followed by soil solarization (SOL) on abundance and infectivity of EPNs using mealworm larva (*Tenebrio molitor*) as a bait. Data were taken every other week throughout the 3 months of corn (*Zea mays*) growth following the preplant treatments. Indigenous EPNs recovered were identified by sequencing the ITS region and matched undescribed species of *Heterorhabditis* in NCBI, labeled as H1, SGgj, and SGmg3. Soil in BO and OR had higher volumetric soil moisture, field capacity, and soil organic matter than BG and SOL ($P \leq 0.05$) in all four trials. In addition, the nematode soil food web at termination of the cover crop, and monthly during corn growth had greater % fungivore (F), fungivore to bacterivore (B) plus F ratio (F/F+B), and channel index ($P \leq 0.05$) than BG and SOL in Trial I, and higher %

omnivores and structure index ($P \leq 0.05$) than BG and SOL in Trial II throughout the corn growing season suggested that no-till with black oat residues was in favor for fungal dominated decomposition path ways in Trial I but lead to more structured soil food web in Trial II. None-the-less, EPN infectivity was greater ($P \leq 0.05$) in BO than in BG and SOL based on field cage assays in both trials. BO may have provided a habitat that enhances EPN infection. Both multivariate canonical analysis conducted for data from Trial I and Trial II suggested that higher EPN infectivity was associated with soil physical properties related to water conservation and improved soil health conditions (higher EI and SI).

In the Oil Radish Experiment, OR increased abundance of *Heterorhabditis* sp. infective juveniles (IJs) as the age of OR increased suggested that OR provided a favorable environment for EPN reproduction. Oil radish growth consistently resulted in higher EPN abundance in OR than in BG and SOL ($P \leq 0.05$) at cover crop termination. However, no-till cover cropping with OR reduce EPNs abundance over time. This could be due to fast decomposition of OR residue that resulted in lack of soil coverage and unfavorable edaphic factors for EPNs. In addition, reduced abundance of herbivorous nematodes and higher occurrence of *Metarhizium anisopliae* from the larva bait assay in OR plots also suggested that allelopathic compounds released from oil radish (*i.e.* isothiocyanates) could have biofumigant effects against EPN infectivities.

In conclusion, this thesis project suggested that BO cover crop in a no-till system provided favorable edaphic factors for EPN infectivity despite lack of ability to enhance IJs abundance of theses nematodes. On the other hand, OR cover cropping enhanced abundance of EPNs, but did not improve EPNs infectivity. Occurrence of *M. anisopliae* on larva bait assay suggested no obvious effect of no-till cover cropping with black oats, and a negative effect of oil radish cover

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CHAPTER 1

LITERATURE REVIEW

1.1 Conservational biological control

Conservational biological control (CBC) is manipulation of the environment to enhance survival, population, epizootics, and effectiveness of natural enemies that are already present in the region to manage pests (Fuxa, 1998; Gurr *et al.*, 2004; Kean *et al.*, 2003; Landis *et al.*, 2000; Pell *et al.*, 2010). In relation to food web dynamics, CBC manages pests by providing an ecosystem that supports top-down regulation of pests via predators or parasitoids (Kean *et al.*, 2003). Classical biological control, involving introduction of alien biological control agents, has a higher risk of being disruptive to vulnerable ecosystems (Ehler, 1998) such as the endemic ecosystems of Hawaii (Funasaki *et al.*, 1988). Furthermore, classical biological control is often challenged by stringent quarantine regulations (Ehler, 1998). Augmentative biological control involves repeated release of a biocontrol agent that might be already present in the region. However, the augmentative biological control approach against crop pests often faces obstacles with inconsistent levels of control (Brust, 1991; Shapiro-Ilan *et al.*, 1999). Some obstacles include 1) losing adaptive traits due to genetic drift from rearing or culturing of the biocontrol agent, resulting in reduced fitness in the natural ecosystem; 2) economic challenges in rearing sufficient numbers of biological control agents for mass release (Ehler, 1998); and the persistence of biological control agents in the field (Susurluk and Ehlers, 2007).

CBC takes advantage of natural enemies that are well-adapted to the specific agroecosystem (Ehler, 1998), which allows the natural enemies to remain persistent in the target agroecosystem

(Shah and Pell, 2003). CBC is a long-term strategy that may become more practical and cost effective than the short-term augmentative biological control (Lewis *et al.*, 1998). Despite these advantages, lack of academic efforts have been dedicated to the study of CBC in comparison to classical and augmentative approaches (Landis *et al.*, 2000; Meyling and Eilenberg, 2007). Therefore, it is well justified to examine the benefits of cover crop in a conservation tillage system on CBC. Specifically, this thesis research focuses on examining the effects of cover crop in the conservation tillage system of no-till on indigenous entomopathogenic nematodes and entomopathogenic fungi in a corn agroecosystem.

1.2 Indigenous entomopathogenic nematodes and entomopathogenic fungi

Entomopathogenic nematodes (EPN) of the genera *Steinernema* and *Heterorhabditis*, and entomopathogenic fungi (EPF) in the order of Hypocerales (also called hyphocetes) of the species *Beauveria bassiana* and *Metarhizium anisopliae*, are soilborn natural enemies of a broad spectrum of arthropod pests and had been suggested to be good candidates for CBC (Stuart *et al.*, 2008; Stuart *et al.*, 2006; Meyling and Eilenberg, 2007). These natural enemies are relevant for CBC studies, since 90% of arthropod pest share some part of their life cycle in the soil (Klingen and Haukeland, 2006; Kaya and Gaugler, 1993). In addition, EPN and EPF are ubiquitous, and can be found naturally present worldwide (Hominick *et al.*, 1996; Stuart *et al.*, 2006).

Hypocerales are ubiquitous in the soil and are associated with insects that come in contact with the soil (Pell *et al.*, 2010). They are usually considered an opportunistic hemibiotrophic fungi. Therefore, they are capable of persisting in the soil as a saprophyte (Pell *et al.*, 2010).

Unlike other entomopathogenic fungi as entomophthoralean fungi that are considered biotrophic (Pell *et al.*, 2010), EPF in Hypocerales can extend their hyphae growth beyond the infected insect cadaver with saprophytic feeding (Klingen and Haukeland, 2006), allowing them to persist in the soil. Hypocerales also have a broad host range from lacking an intimate relationship with its host, killing its host by overwhelming the host immune system with toxin production (Shah and Pell, 2003). Although EPF tend to occupy the soil surface, they have been detected at soil depths of 30 cm deep (Klingen and Haukeland, 2006).

EPNs of the genera *Steinernema* and *Heterorhabditis* also have a broad host range on nearly all insect orders under laboratory conditions (Kaya and Gaugler, 1993) and do not possess any mammalian pathogenicity (Brust, 1991). EPNs also lack an intimate parasitic relationship with their arthropod hosts, thus they have a broad insect host range (Kaya and Gaugler, 1993). However, there is evidence of limited insect infection in field conditions (Kaya and Gaugler, 1993). EPNs are associated with mutualistic bacteria that are the primary agent in killing the insect host (Ehler, 1990). The bacteria cause septicemia within 24-48 hours after infecting the insect hosts (Adams and Nguyen, 2001; Kaya and Gaugler, 1993). *Steinernema* carries the mutualistic bacteria of the genus *Xenorhabdus*, whereas *Heterorhabditis* carries the mutualistic bacteria of the genus *Photorhabdus* in its intestinal tract (Kaya and Gaugler, 1993). Infective juvenile (IJ), also known as dauer larvae or third-stage juvenile of the EPN, penetrates into the insect host through wounds, natural openings (mouth, anus, or spiracles), or sometimes direct penetration of the cuticle (Glazer and Lewis, 2000) with the assistance of dorsal tooth in *Heterorhabditis* (Kaya and Gaugler, 1993). Once successfully entered into the insect host, they release the mutualistic bacteria, while secreting proteinaceous substances to neutralize the host's

immune system and nervous system to assist in bacterial colonization (Glazer and Lewis, 2000). The bacteria not only kill the host, but produce antibiotics to protect the cadaver from secondary infection of other microorganisms, digest the host tissue to make nutrients more available, and become a source of food for the bacterivorous EPN (Kaya and Gaugler, 1993).

Unlike Hypocerales, EPNs are obligate pathogens of insects, requiring arthropods for reproduction, often described as recycling (Kaya and Gaugler, 1993). Many EPN species share a different host finding infection strategy from Hypocerales described as “cruiser” foraging behavior (*i.e.* *Heterorhabditis* spp. and *S. glaseri*), in which nematodes crawl towards their host (Campbell and Lewis, 2002). In contrast, Hypocerales require their non-mobile spores to come in contact with the insect host (Hummel *et al.*, 2002; Barbercheck 1992). However, other EPN species (*i.e.* *S. carpocapsae*) have a non-mobile host finding strategy called “ambush” foraging behavior, remaining sedentary in the soil until a mobile host comes in contact with the EPN (Kaya and Gaugler, 1993). Ambushers are believed to take a more energy conserving approach to host finding, but are limited to infect mobile insect host near the soil surface. Whereas cruisers have the advantage of finding immobile hosts and hosts at a deeper soil profiles (Kaya and Gaugler, 1993). This research focused on studying an indigenous *Heterorhabditis* sp. detected in field sites of this thesis research.

1.3 Improving conditions with conservation tillage

Much of the literature suggests that conservation tillage can improve conditions for entomopathogens. Conservation tillage was developed in response to the detriments of

conventional tillage. Conventional tillage involves inverting the topsoil with a plow at least to a depth of 20 cm (Kassam *et al.*, 2009), which is generally practiced by using a moldboard plow, followed by secondary cultivation to form a planting bed (Holland, 2004). Tilling was widely adopted by farmers because of the improvement of soil aeration, reduction of initial pest populations, creation of planting bed (Triplett and Dick, 2008), and post-emergent weed control (Lal *et al.*, 2007). Despite these benefits, intensive farming with conventional tillage has led to physical and biological soil degradation, reduced soil productivity, increased soil erosion (Kassam *et al.*, 2009), and desertification, resulting in catastrophic disasters like the dust bowl in the Midwest of the United States during the 1930s (Holland 2004; Giller *et al.*, 2015). As dust storms notoriously called “black blizzards” (Bavey *et al.*, 2011) swept through the Midwest, many agriculturists challenged the age-old practice of tillage.

Due to the adverse effects of tillage, especially during the 1930s, conservation tillage strategies were proposed by an extension worker in Ohio, Edward Faulkner, who published the book “Plowman’s Folly” in 1942, and pioneered the no-till movement (Lal *et al.*, 2007). Largely due to environmental and human tragedies of the dust bowl, the U.S. federal government took an initiative for soil conservation as President Roosevelt on February 26, 1947 wrote: “The nation that destroys its soil destroys itself” (Bavey *et al.*, 2011). Since the 1950s, growers have been transitioning from the moldboard plow to reduced soil disturbance (Lal *et al.*, 2007), which lead to the term “reduced tillage” in the 1960s defined by the Conservation Technology Information Center (CTIC) as tillage that maintains 15-30% soil surface coverage by crop residue (Mitchell *et al.*, 2009). In 1984, the U.S. Soil Conservation Service defined the approach of reducing soil disturbance with the term “conservation tillage” as any tillage system that aims at reducing water

erosion by maintaining 30% crop residue coverage on soil surface or 1,120 kg/ha (Reeder, 2000). Conservation tillage has gain grounds throughout the 1960s and 1970s through the availability of effective herbicides, no-till planters, and government policy incentives (Giller *et al.*, 2015). In the 1990's, genetically-modified crops with herbicide resistant traits helped to expand conservation tillage acceptance by growers (Giller *et al.*, 2015). Besides the U.S. mainland, conservation tillage has received international acceptance in Australia, South America, Canada (Triplett and Dick, 2008), Europe (Holland, 2004), Africa (Giller *et al.*, 2015), Brazil, and Argentina (Brady and Weil, 2010), as many have recognized the benefits of this cultural practice expanding no-till to more than 110 million ha world-wide (Derpsch *et al.*, 2010). In fact, Argentina and southern Brazil are making significant progress in expanding no-till acreages as thousands of small-scale soybean and corn farmers are adopting cover-crop-based no-till agriculture with animal tractors and small tractors (Brady and Weil, 2010).

Since conservation tillage is loosely defined, there are many approaches including no-till, strip-till, ridge-till, and mulch-till. In this study, the no-till approach will be examined. No-till, also known as zero till, direct drilling (Holland, 2004) and chemical till (Lal *et al.*, 2007), can be described as planting directly into the previous crop residue in the absence of any tillage. No-till has become the most popular conservation tillage approach due to the availability of herbicides and genetically modified herbicide tolerant crops for post-emergent weed control, drastically claiming new acreage in agriculture production (Lal *et al.*, 2007). Benefits associated with no-till practices include:

1) Improve in soil health: Reduction in soil disturbance greatly improves soil health, also referred to as soil quality. A healthy soil can be determined by its physical, biological,

and chemical properties (Havlin *et al.*, 2014) and can be defined as a soil providing plant anchorage; nutrient supply and recycling; retaining optimal plant available water; supporting soil food webs; maintaining microbial diversity, disease suppression; remediating pollutants; and sequestering heavy metals (Wang and McSorley, 2005).

Biological activities are known to be enhanced by conservation tillage from surface residue lowering soil temperatures, increasing soil water conservation, and providing organic matter for microbial decomposition (Havlin *et al.*, 2014). Due to the enhancement of biological activity, soil aggregation (Brady and Weil, 2010; Gebhardt *et al.*, 1985) and nutrient cycling of nitrogen, sulfur, and phosphorus is also improved in no-till systems (Havlin *et al.*, 2014). Crop residue from practicing no-till provides a more stable environment for diverse groups of natural enemies of pests, for example beetles and spiders (Schmidt *et al.*, 2004). Besides enhancing soil macro-fauna, organic residue in no-till provides a favorable environment for beneficial fungi. For example, no-till has been reported to improve colonization of mutualistic mycorrhizae due to reduced disturbance of the soil providing an environment for extraradical hyphae from host plants to become a main source of inoculum (Kabir, 2005). Proliferation of mycorrhizal fungi has been reported to protect plants from root pathogens (Thygesen *et al.*, 2004), increasing water use efficiency (Caravaca *et al.*, 2004), while improving soil structure (Bethlenfalvay and Barea 1994). There have also been observations of no-till enhancing entomopathogenic nematodes and fungi which will be discussed later in this thesis.

2) *Reduce soil erosion*: Topsoil is the most fertile layer of arable land. When intensive tillage is practiced, topsoil erosion can result in exposure of less productive subsoil, which

have poor physical, biological, and chemical properties with low organic matter, microbial activity, nutrient supply, infiltration, and plant available water resulting in reduced productivity (Havlin *et al.*, 2014). No-till can greatly reduce erosion of the top soil by maintaining a vegetative cover over the soil, helping to maintain the soil structure and aggregation (Pimentel, 1995), allowing more water infiltration (Triplett and Dick, 2008), decreasing soil detachment by water and wind (Havlin *et al.*, 2014) and improve aggregate stability from increase of soil organic matter (Peigné, 2007; Brady and Weil, 2010). Growing cover crops in a fallow field can also increase surface residue that is compatible with soil health management (Brady and Weil, 2010).

3) *Increase soil organic matter and nutrient supply*: The practice of conservation tillage increases soil organic matter (SOM) in the top 5 cm of soil (Havlin *et al.*, 2014) progressively over time (Reichert *et al.*, 2016). This is due to the delay of decomposition of organic matter in conservation tillage compared to faster decomposition of residue homogenized in the plowed layer during tillage (Brady and Weil, 2010; Zotarelli *et al.*, 2007). Faster decomposition is due to mainly two factors: 1) tillage exposes the SOM to oxygen resulting in oxidative loss (Brady and Weil, 2010; Beare *et al.*, 1994) and 2) aerobic microbial decomposition in the soil increases organic matter mineralization (Havlin *et al.*, 2014). Conservation tillage increased soil carbon by 8% compared to conventional tillage in the U.K., as significant differences can be detected after 4 years of conservation tillage (Holland, 2004). Soil organic matter analysis from a no-till plot after seven years of consecutive rotation between cash crops and cover crops at Poamoho Experiment Station,

University of Hawaii showed 14% increase in SOM in an Oxisol. This thesis research was based at this long-term no-till field.

SOM improvement has been considered the most critical soil physical property that affects soil productivity (Havlin *et al.*, 2014; Ismail *et al.*, 1994). As SOM increases, soil aggregate stability is improved by 1) forming bridges between soil particles of silicate clays and iron and aluminum oxides; and 2) providing food for fungi and bacteria which exude polysaccharides and organic compounds that form sticky networks to bind soil particles together (Brady and Weil, 2010). In addition, SOM increases nutrient balance in the soil (especially of nitrogen, sulfur, and phosphorus) by 1) influencing soil microorganisms involved in mineralization or immobilization of soil nutrients (Havlin *et al.*, 2014); 2) contributing to 50-90% of soil cation exchange capacity that helps to exchange many important plant nutrients (Ca^{2+} , Mg^{2+} , K^+ , NH_3^+ , etc.) with plant roots; and 3) chelating micronutrients to make them more readily available for plants (Brady and Weil, 2010).

4) *Increase plant-available water*: Interestingly, in studies conducted in a dry climate under rain-fed agriculture, no-till increased crop yields by 7.3% when integrated with cover cropping (Pittelkow *et al.*, 2015), suggesting that no-till provides plants an advantage in a water stressed environment when cover crop residue is integrated. Hill (1990) also reported that no-till retained more moistures at higher water potentials (3.9 to 40 kPa) than conventional tillage, while conventional tillage can retain more moistures at lower water potentials (0-2 kPa) in a Hapludults from Maryland. Similar result was observed by Tormena *et al.*, (1999) in Hapludox in Brazil, and by Arshad and Azooz (1995) in a Canadian Cryoboralfs. One reason is that tillage increases macroporosity, but does not

preserve its microporosity and capillary pores responsible for water holding capacity (Arshad and Azooz, 1995).

Surface organic mulch associated with conservation tillage systems reduce soil temperature, thus reducing soil moisture evaporation (Havlin *et al.*, 2014). Also, organic mulch increases SOM; leads to better soil aggregate and stability; and improves water infiltration and water holding capacity (Gebhardt *et al.*, 1985; Pittelkow *et al.*, 2015). In addition, organic mulch conserved in conservation tillage can 1) serve as a buffer to raindrop impact, and 2) maintaining higher water infiltration rates (Havlin *et al.*, 2014) and reduce water runoff (Gebhardt *et al.*, 1985), therefore increasing water use efficiency. Therefore, crops in areas that experience water stress would benefit from conservation tillage.

On the other hand, plant available water is lost in conventional tillage. This is because tillage destroys the structure of soil aggregates resulting in dispersed clay that clogs soil pores and forms a surface seal. When this surface seal dries after precipitation, it can form a hard soil crust in favor of water runoff (Brady and Weil, 2010).

1.4 Overcoming challenges of no-till

According to a comprehensive meta-analysis of 610 studies from 63 countries and 48 crops of side-by-side yield comparisons with conventional tillage, no-till alone has resulted in 9.9% yield reduction (Pittelkow *et al.*, 2015). However, when residue retention and crop rotation were applied, yields improved, with only marginally lower yields of 2.5% from conventional tillage (Pittelkow *et al.*, 2015). Common challenges of no-till consist of weed management of perennial

weeds (Derksen *et al.*, 1996) and persistence of plant pathogens (Sturz *et al.*, 1997). Many have suggested crop rotation to minimize persistence of diseases (Cook and Haglung 1991; Bockus and Shroyer 1998; Sturz *et al.*, 1997; Dill-Macky and Jones 2000). Therefore, no-till should not be practiced alone. While no-till increased yields 7.3% above conventional tillage when accompanied with residue retention and crop rotation in a dry climate, yields declined 11.9% below conventional tillage when no-till stood alone (Pittelkow *et al.*, 2015), suggesting that residue retention and crop rotation has a synergetic relationship with no-till in improving yields under dry climates. It is also interesting to note that yield improvement tends to become more apparent in dryer climates (He *et al.*, 2009).

Integrating residue retention and crop rotation together is known as conservation agriculture (CA). Therefore, incorporating CA principles in conservation tillage may optimize the benefits and overcome the challenges of no-till farming. The Food and Agriculture Organization of the United Nations (FAO) list three CA principles: 1) minimal soil disturbance; 2) continuous soil cover; and 3) crop rotation (Jat *et al.*, 2013). USDA Natural Resource Conservation Services (NRCS) have also promoted the principle of continuous living roots (NRCS, 2013) to provide nutrients via root exudates for beneficial soil microorganisms that feed on carbohydrates, organic acids, amino acids, phenolic compounds, nucleotides, and vitamins provided by the roots (Klingen and Haukeland, 2006; Brady and Weil, 2010).

1.5 Improving no-till with cover cropping

Incorporating cover cropping into the farm management program can serve to provide continuous soil cover, crop rotation, and continuous living roots in a CA system. Cover crops can

be referred to as crops grown 1) between cash crop cycles followed by soil incorporation, often this is also known as green manure (Wang *et al.*, 2001); or 2) between cash crop cycles but its residues will not be soil incorporated after termination, this is known as surface organic mulch (Quintanilla-Tornel *et al.*, 2016); 3) between plants in an orchard or vineyard often known as ground cover (Fageria *et al.*, 2005); or 4) between cash crop planting rows in a row crop system, this is known as living mulch (Wang *et al.*, 2011; Hartwig and Ammon 2002). In this thesis research, cover crops will be used as surface organic mulch in a no-till cropping system.

Terminating cover crop in a no-till cropping system gained its popularity in sustainable agriculture research in 1970s (Gallaher, 2002). Over the years, scientists reported “Minimum Tillage, Multiple Cropping System” helps to improve soil coverage (Lu *et al.*, 2000), suppress weeds (Lu *et al.*, 2000; Yenish *et al.*, 1996; Burgos and Talbert 1996), decrease plant pathogen infestation (Abawi and Widmer, 2000), reduce soil erosion (Lu *et al.*, 2000, Havlin *et al.*, 2014), mitigate nitrate leaching (Constantin *et al.*, 2010), reduce soil compaction (Calonego and Rosolem 2010), improve soil aggregation (Villamil *et al.*, 2006), conserve soil moistures (Lu *et al.*, 2000; Daniel *et al.*, 1999), increase soil organic matter (Amado *et al.*, 2006; Bayer *et al.*, 2001; Villamil *et al.*, 2006; Calegari *et al.*, 2008), enhance microbial activity (Abawai and Widmer, 2000), raise soil nitrogen pools (Bayer *et al.*, 2001) and enhance soil nutrient cycling (Lu *et al.*, 2000). In addition, cover cropping can also enhance suppressive activities of natural enemies of crop pests (Klingen and Haukeland 2006; Pullaro *et al.*, 2006). For example, white clover (*Trifolium repens* L.), improved persistence of the entomopathogenic fungi *Beauveria bassiana* in the field (Shapiro-Ilan *et al.*, 2012). Quitanella *et al.* (2016) demonstrated that sunn hemp (*Crotalaria juncea*) terminated in a no-till cropping system resulted in less thrips and leaf

miners damage on green onion. This approach of pest management using cover crop in a conservation tillage system to provide habitats favoring conservation of natural enemies in an agroecosystem is in line with conservation biological control (CBC) (Landis *et al.*, 2000).

1.6 Effects of no-till practice on soil-born entomopathogens

Keller *et al.* (2003) found higher abundance of *M. anisopliae* in meadows compared to the adjacent arable land. Hummel *et al.* (2002) found conservation tillage increased abundance of EPN and EPF compared to conventional tillage. Sosa-Gomez and Moscardi (1994) found abundance of *B. bassiana*, *M. anisopliae*, and *Paecilomyces* to be greater in no-till soybean and wheat double cropping system than conventional tillage. Bing and Lewis (1993) found higher colony forming units (CFU) of *B. bassiana* in no-till than conventional tillage; however, it was only observed during the first year of the study, suggesting that there may be other factors affecting CFU detections of this EPF. Burst (1991) found that no-till with the presence of weeds increased the detection of *H. bacteriophora* from conventional tillage from a corn field infested with spotted cucumber beetle (*Diabrotica undecimpunctata*). However, there was no interaction between no-till practice and the presence of weeds in the detection levels of EPN.

Many factors are responsible for improving conditions for entomopathogens in reduced tillage. One factor is reduced soil disturbance. Tillage can expose EPN to ultraviolet light and desiccation which is harmful to EPN (Kaya, 1990). No-till practice allows EPF to remain close to the soil surface increasing the chances of contact of insect hosts (Pell *et al.*, 2010). This may also be true for EPN, as they tend to stay near the soil surface (Moyle and Kaya, 1981) as *S.*

carpocapsae and *H. bacteriophora* are found in the top 1-2 cm. However, *S. carpocapsae* have been found as deep as 35 cm (Lewis, 2002). Higher soil organic matter is also a factor associated with no-till that could have an effect on entomopathogens. SOM increases density of arthropod hosts and predators and antagonistic organisms of entomopathogens (Klingen and Haukeland, 2006). Despite reports of SOM being detrimental to EPF, *B. bassiana* has been associated with undisturbed soil high in organic matter (Mietkiewski *et al.*, 1997). Improved soil water properties are also associated with reduced tillage which can affect entomopathogens. Hummel *et al.*, (2002) suggested that higher soil moisture may play an important role in conservation tillage to enhance entomopathogens. One explanation for enhancement of entomopathogens by higher soil moisture could be the reduction of nematode mortality from desiccation in the top 10 cm of soil in which majority of EPN are present (Burst, 1991). Soil moisture is also important for host finding, as water film surrounding soil particles is required for movement of EPN (Klingen and Haukeland, 2006; Barbercheck, 1992). As the water film becomes thinner, cohesive force increases between the nematode and soil particle resulting in increased resistance to movement (Wallace, 1971). Once the water film becomes 1 μm thick, the surface tension forces are too great for the nematode to move (Barbercheck, 1992). Grant and Villani (2003) observed that virulence of *H. bacteriophora*, *S. glaseri*, *S. feltiae*, and *S. carpocapsae*, increased with increasing soil moisture content in sandy loam soils. On the other hand, although Burst (1991) observed enhancement of EPN infections in no-till plots, lack of irrigation did not affect the infectivity of the endemic *H. heliothidis*, suggesting that that other factors like reduced soil disturbance, higher organic matter, and higher abundance of alternative host could play a larger role in enhancing EPN infectivity in the no-till plots. Yields were not affected by irrigation in

Burst's (1991) study suggesting that there may not have been a significant difference in plant available water and soil moisture. Thus, effect of soil moisture on EPN infectivity was inconclusive (Burst, 1991). Soil moisture can also have a negative effect on EPN when it is too high. Anaerobic conditions from saturated soil can reduce survival and infectivity of *S. carpocapsae* and *S. glaseri* (Barbercheck, 1992). In addition, as the soil becomes saturated, there will be lack of surface tension between the nematode and soil particle (Wallace, 1971). Soil moisture may also have a negative effect on EPF, as it has been reported that *B. bassiana* conidia half-life is reduced as the soil became moister starting from permanent wilting point (-150 kPa) (Studdert *et al.*, 1990). It is hypothesized that higher soil moisture maintained in no-till cover cropping system would increase the abundance and infectivity of EPF as long as anaerobic conditions are avoided.

Organic mulch left over from previous crop may also play an important role in affecting entomopathogens in a no-till system. Organic mulch has been reported to attract predators and parasitoids (Schmidt *et al.*, 2004) which can help to disperse the insect host of entomopathogens and increase transmission of EPF (Roy and Pell, 2000). In addition, organic mulch may also enhance conditions for collembolans, which have been observed vectoring *B. bassiana*, *B. brongniartii* and *M. anisopliae* causing mortality to the target host (Dromph, 2003). Since EPN are obligate parasites, alternative host are important for reproduction and persistence of these nematodes. Kaya and Gaugler (1993) suggest that cultural practices that conserve plant residue like no-till can help provide alternative arthropod hosts for survival and recycling of EPN. Non-phytophagous arthropods living in the organic mulch may serve as alternative hosts to assist in reproduction and persistence of EPN. For example, the beetle, *Strophosoma faber* have been

found to enhance EPN infections of the turf pest, the European June beetle (*Amphimallon solstitiale*) (Kowalska, 2000). An organic mulch can also enhance conditions for entomopathogens by conserving soil moisture and lowering temperatures. Shapiro-Ilan *et al.* (1999) discovered that *S. carpocapsae* applied to tilled plots with soybean stubble residue on the soil surface persisted significantly better (above 85%) than without residue (below 35%) as the crop residue may have protected *S. carpocapsae* from desiccation and ultraviolet light. Reduced tillage tend to have less fluctuations in temperature and moisture (Stuart *et al.*, 2006), as crop residue protects the soil surface for solar radiation and evaporation (Griffith *et al.*, 1986). Soil temperatures above 37°C are known to kill EPNs (Stuart *et al.*, 2006).

1.7 Effect of cover cropping on entomopathogens

Planting cover crop alone can improve conditions for entomopathogens by providing 1) continuous living roots, and 2) continuous insect hosts. Roots help in migration (Klingen and Haukeland, 2006) and attraction of EPNs to plants attacked by insects (Van Tol *et al.*, 2001; Ali *et al.*, 2012; Lei *et al.*, 1992). Root exudates of brassicaceous plants (*Eruca vesicaria sativa* and *Barbarea vulgaris* T. *cylindrosporum*) have been shown to enhance germination and survival of *M. anisopliae* in the rhizosphere (Klingen *et al.*, 2002). Therefore, utilizing cover crops could support a CBC approach in providing continuous living roots that may assist in attracting EPN and persistence of EPF. High occurrence of EPF was found under rowan trees from the continuous presence of apple fruit moth (*Argyresthia conjugella*) larvae and the absence of pesticides (Vänninen *et al.*, 1989). In addition, small insect larvae associated with cruciferous crops like *Delia radicum* (cabbage fly), *Meligethes stephens* (pollen beetle), *Ceutorrhynchus*

assimilis (cabbage seed weevil), and *Mamestra brassicae* (cabbage moth) can assist in reproduction of EPN (Nielsen and Philipsen, 2004). However, this phenomenon has not been confirmed in field conditions. Lei *et al.* (1992) observed an EPN, *H. zealandica* was attracted to roots and germinating seeds of head cabbage and radish. Therefore, one of the objective of this thesis is to examine oilseed radish (*Raphanus sativus* L. ssp. *oleiferus* cv. Sodbuster) as a cover crop followed by no-till practice in conserving and augmenting indigenous EPN populations in a corn agroecosystem. It is unclear whether oil radish would attract EPN via its leachate or insect hosts.

Abiotic factors associated with cover crops enhancing entomopathogens are related to soil surface coverage: 1) reduced soil temperatures and 2) increased soil moisture from bare fallow ground. Hummel *et al.* (2002) found that the greatest detection of entomopathogens came from plots with the lowest soil temperatures from living mulch or from residue of cover crops, while lowest detection of entomopathogens came from plots with no soil cover or with black plastic mulch having the highest soil temperatures at a depth range of 2-14 cm.

In this thesis, black oat (*Avena strigose*) and oilseed radish (*Raphanus sativus*) were selected as candidates for cover cropping as they might provide different approaches in enhancing entomopathogens. *Avena strigose* is a grass that can produce crop residue with high carbon to nitrogen (C:N) ratios with 1.5% tissue nitrogen at cereal heading (Gaskell *et al.*, 2011). Residue with a high C:N ratio should persist longer in the field as an organic mulch due to slower decomposition rates. Oil radish on the other hand has a lower C: N ratio of 2.5% to 3% tissue nitrogen (Gaskell *et al.*, 2011) and its residues should decompose quickly. Therefore, oil radish residues would provide less soil coverage compared to black oats or other grasses. However, oil

radish can attract many insects and improve conditions for alternate host infections of entomopathogens, resulting with in-field augmentation before the main crop, corn (*Zea mays*) is planted in the field. Therefore, in this thesis project, the performance of two distinct cover crops in enhancing population densities of entomopathogens were compared.

1.8 Objectives and hypotheses

The overall goal of this research project is to assess the effects of no-till cover cropping on entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) in a corn agroecosystem. Different types of cover crops might provide different ecosystem services to enhance entomopathogens to achieve conservation biological control (CBC). The aim of this thesis is to understand the relationship of entomopathogens with various soil edaphic factors associated with no-till (NT) and cover cropping in aims of improving CBC of entomopathogens in corn agroecosystem. Specific objectives of this project were to:

Objective 1: Determine if NT cover cropping with black oats can enhance entomopathogens.

Ho 1: No-till cover cropping with a high C: N cover crop such as black oat would provide an organic mulch layer in a less disturbed soil community of higher soil organic matter and improved soil water conservation. All of which could contribute to improved edaphic factors to enhance of entomopathogens.

Ho 2: Soil solarization would partially sterilized the top soil layer and reduce entomopathogens populations which would reduce crop yields.

Ho 3: Improvement of water conservation and enhancement of entomopathogens in NT could lead to increase in corn yield.

Ho 4: Soil food web and edaphic factors of BO is correlate with enhanced entomopathogens activity.

Objective 2: Determine if NT cover cropping with oil radish can enhance entomopathogens.

Ho 5: Oil radish will increase abundance of EPN in the soil.

Ho 6: Oil radish can attract EPNs through herbivore induced volatiles (HIPVs) when tissue is macerated at termination.

Ho 7: Higher abundance of EPNs from oil radish will lead to enhance infectivity of EPN.

Ho 8: Enhancement of EPN by oil radish in NT system could lead to increase in corn yield.

Ho 9: Soil food web and edaphic factors of OR will correlate with entomopathogen activity.

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CHAPTER 2

ENHANCEMENT OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES AND ENTOMOPATHOGENIC FUNGI BY NO-TILL COVER CROPPING WITH BLACK OAT (*AVENA STRIGOSE*) IN A CORN (*ZEA MAYS*) AGROECOSYSTEM

Abstract

Two field trials were conducted to examine if conservation agriculture through cover cropping of black oats (*Avena strigose*) followed by no-till would enhance entomopathogenic nematodes (EPNs) and entomopathogenic fungi (EPF). EPN and EPF in a corn (*Zea mays*) agroecosystem were monitored in three pre-plant treatments: 1) black oat (BO) as a cover crop followed by no-till planting, 2) bare ground (BG) followed by conventional tillage, and 3) conventional tillage followed by soil solarization (SOL). No-till planting with BO had higher volumetric soil moisture, field capacity, soil organic matter, greater % fungivore, fungivore to bacterivore ratio (F/F+B) and channel index ($P \leq 0.05$) than BG and SOL in Trial I, and higher % omnivores and structure index ($P \leq 0.05$) than BG and SOL in Trial II. EPN infectivity was greater ($P \leq 0.05$) in BO than BG and SOL based on field cage assays but not laboratory assays. Abundance of *Heterorhabditis* infective juveniles (IJs) extracted from the soil were also higher in BO than SOL ($P \leq 0.05$), but was not different from that in the BG in Trial I, but were higher than BG in Trial II. A multivariate canonical analysis suggested that tritrophic interactions among EPNs, thrips (*Frankliniella williamsi*) and corn growth occurred in Trial I, though not in Trial II due to lower thrips count. None-the-less, BO is consistently associated with more structured soil food web and higher *Heterorhabditis* abundance and infectivity.

Keywords: Conservation agriculture, conservation biological control, *Heterorhabditis*, *Metarhizium anisopliae*, canonical correlation analysis, solarization.

2.1 Introduction

Entomopathogenic nematodes (EPN) in the genera *Steinernema* and *Heterorhabditis*, and entomopathogenic fungi (EPF) in the order of Hypocerales (also known as hyphocetes) such as *Beauveria bassiana* and *Metarhizium anisopliae* occur commonly as biological control agents against many insect pests, and are applied through augmentative release for insect management since 1923 by Rudolf Glaser to manage populations of Japanese beetles (*Popillia japonica*) (Poinar and Grewal, 2012). This approach has successfully controlled wide spectrum of insect pests on high value crops (Shapiro-Ilan *et al.*, 2002). However, augmentative release of EPNs against crop pests often face obstacles like inconsistent performance in the field (Brust, 1991; Lewis *et al.*, 1998; Shapiro-Ilan *et al.*, 1999) often requiring reapplications (Shapiro-Ilan *et al.*, 2012). Obstacles of entomopathogen field release include 1) losing adaptive traits to the agroecosystem due to genetic drift from rearing or culturing in the laboratory, resulted in reduced fitness in the field; 2) rearing sufficient numbers for mass release – generally 1 to 5 billion infective juveniles (IJs) per hectare for EPNs (Ehler, 1998; Susurluk and Ehlers, 2007) and 5 billion conidia per hectare for EPF (Bateman and Chapple, 2001); 3) persisting in the field (Susurluk and Ehlers, 2007); and 4) introducing alien entomopathogens species due to quarantine restrictions in certain areas such as Hawaii (Myers *et al.*, 2015) and the United Kingdom (Kaya and Gaugler, 1993). Therefore, conservation of indigenous or naturally occurring

entomopathogens has been suggested as a more practical and cost effective biological control approach known as conservation biological control (CBC) (Stuart *et al.*, 2006).

Cultural practices that have been recognized to facilitate CBC of entomopathogens are conservation tillage and cover cropping (Hummel *et al.*, 2002). There have been multiple reports of conservation tillage improving conditions for entomopathogens. For examples, *H. bacteriophora* prevalence decreased following a tillage (Susurluk and Ehlers, 2007); no-till with the presence of weeds increased the detection of *H. bacteriophora* compared to conventional tillage in a corn (*Zea mays*) field infested with spotted cucumber beetle (*Diabrotica undecimpunctata*) (Burst, 1991). Similarly, abundance of EPF such as *M. anisopliae* was higher in the meadows than the adjacent arable land (Keller *et al.*, 2003), *B. bassiana*, *M. anisopliae*, and *Paecilomyces* spp. were greater in soybean (*Glycine max*) and wheat (*Triticum*) double cropping no-till system than that in conventional tillage system (Sosa-Gomez and Moscardi, 1994); and colony forming units (CFU) of *B. bassiana* was higher in no-till than conventional tillage (Bing and Lewis, 1993).

Growing cover crops have also been reported to improve field conditions favorable for entomopathogens. Entomopathogen activity was greatest in conservation tillage treatment with cover cropping of fall-planted hairy vetch (*Vicia villosa*) and a living mulch of red clover (Hummel *et al.*, 2002). *Steinernema carpocapsae* migrated farther in the center row of corn inter-planted in dicot-monocot cover crop mix as a living mulch system (Jabbour and Barbercheck, 2008). Persistence of *H. bacteriophora* was greatest in a field of beans followed by a rotation of wheat with red clover as a cover crop (Susurluk and Ehlers, 2007). Detection of *S.*

carpocapsae was also significantly improved in plots with no-till wheat cover crop in the fall compared to tilled plots that were chisel plowed and disked (Millar and Barbercheck, 2002).

Organic mulch from residue of cover crop may be an important factor to improving soil conditions for entomopathogens. Organic mulch has been reported to attract predators and parasitoids (Schmidt *et al.*, 2004) which can help to disperse the insect host of entomopathogens and increase transmission of EPF (Roy and Pell, 2000). In addition, organic mulch may also enhance conditions for collembolans, which have been observed vectoring *B. bassiana*, *B. brongniartii* and *M. anisopliae* causing mortality to the target host (Dromph, 2003). Since EPN are obligate parasites, alternative host are important for reproduction and persistence of these nematodes. Kaya and Gaugler (1993) suggested that cultural practices that conserve plant residue like no-till can help provide alternative hosts for survival and recycling of EPN. Non-phytophagous arthropods living in the organic mulch may serve as alternative hosts to assist in reproduction and persistence of EPN. For example, the beetle, *Strophosoma faber* has been found to enhance EPN infections of the turf pest, the European June beetle (*Amphimallon solstitiale*) (Kowalska, 2000). An organic mulch can also enhance conditions for entomopathogens by conserving soil moisture and lowering temperatures. Shapiro *et al.* (1999) discovered that *S. carpocapsae* applied to tilled plots with soybean stubble residue on the soil surface persisted better (above 85% survival rate) than without residue (below 35% survival rate) as crop residue may have protected *S. carpocapsae* from desiccation and ultraviolet light. Reduced tillage is known to create less fluctuations in soil temperature and moisture (Stuart *et al.*, 2006), as crop residue protects the soil surface from solar radiation and evaporation (Griffith *et al.*, 1986).

While there are many reports on the positive effects of conservation tillage and cover cropping on entomopathogens, using cultural practices to enhance entomopathogens for conservation biological control is largely undeveloped and speculative (Stuart *et al.*, 2008). There are even fewer studies on adequately linking interactions of naturally occurring entomopathogens to various soil edaphic factors (Klingen and Haukeland, 2006) and soil health conditions in a no-till cover cropping system due to the difficulty of multiple direct and indirect biotic and abiotic effects in the field (Barbercheck, 1992; Stuart *et al.*, 2006). The use of multivariate canonical correspondence analysis (CCA) could assist in observing these multiple interactions in the field. Therefore, a field study was conducted to examine the effect of no-till cover cropping with black oat (*Avena stringosa*) on the indigenous population of entomopathogens. Black oat was selected due to its high carbon content that can persist as a surface organic mulch over a cash crop growing period of 3 months.

Specific objectives of this research were to determine if cover cropping with black oat followed by no-till practice would be a feasible conservation biological control strategy by 1) improving soil physical properties in favor of EPN and EPF persistence throughout one cropping cycle of corn; 2) increasing EPN and EPF abundance and infectivity over time, and 3) preserving a healthy soil food web that could foster EPN abundance and infectivity.

2.2 Materials and Methods

Trial I: A field trial was conducted at Poamoho Experiment Station, University of Hawaii (2132° 9.6756'' N, 158 5° 21.8796'' W), Waialua, Oahu. The soil type at the site is a well-drained

silty clay Oxisol (Wahiawa series, very fine, kaolinitic, isohyperthermic, rhodic haplustox) (Ikawa *et al.*, 1985) with an average pH of 6.6. Three pre-plant soil treatments established prior to corn planting were black oat (*Avena strigosa*) cover crop in a no-till system (BO), soil solarization (SOL) and bare ground (BG), each with 4 replications, arranged in a randomized complete block design (RCBD). BO was established in field plots with 7 years of no-till cover crop-vegetable crop rotation practice. Black oat was sown on 20 May, 2016 at 32 kg seeds/ha and grown as a cover crop for 3 months. The cover crop was then terminated on 8 July, 2016 using a flail mower. Solarization was by covering of tilled soil with 1.2-m wide, 25- μ m thick, uv stabilized, low density transparent polyethylene mulch (ISO Poly Firms, Inc., Gary Court, SC). Soil solarization treatment was used as a negative control as it was expected to kill majority of the entomopathogens after reaching a lethal temperature of 37 °C (Stuart *et al.*, 2006). Soil temperature was measured with temperature probes (WatchDog B-series button data logger, Spectrum® technologies, Aurora, IL) and buried at a depth of 5 and 15 cm in one plot of each treatment during the cover cropping period. Soil in the BG treatment remained fallow with weeds followed by rotor tilling prior to corn planting. Thus, BG served as the conventional tillage control. Each treatment plot was 3.7 \times 11 m² in size. Six rows of corn were planted on 14 July, 2016 in 35.5-cm row spacing, 23 cm between plants within row at a rate of 15,240 seeds/ha. The crop was fertilized at a rate of 130 kg of nitrogen, 56.7 kg of phosphorus, and 108 kg of potassium per ha. Crops were drip irrigated uniformly throughout the field based on the crop requirement. The experiment was terminated at 12 weeks after corn planting.

Trial II: The experiment was repeated in the same field plot from December 2016 to May 2017. Black oat cover crop in the no-till plots was grown from 8 December, 2016 to 23 February,

2017. Due to concerns with contaminating the sterilized soil from Trial I, solarized plots in Trial II were not tilled and were tarped with solarization mulch from December to February. Corn was planted on 2 March, 2017 and harvested on 25 May, 2017.

Soil analysis: Soil samples were systematically collected from 6 cores from the top 10-cm of soil per plot using a GroundShark Shovel (W.W. Manufacturing, Inc., Bridgeton, NJ) at corn planting, and at 2 and 3 months after planting. Soil cores from each plot were composited into a plastic bag, and transported to the laboratory to process for nematode extraction, gravimetric soil moistures measurement, and larvae bait assay. The first and last soil samples at planting and harvest were submitted to Agriculture Diagnostic Services Center (ADSC) at the University of Hawaii at Manoa for analysis of total carbon by LECO TruSpec CN (LECO Corporation, Saint Joseph, MI) to estimate SOM.

Nematode analysis: A 250 cm³ subsample was taken from soil samples for nematode extraction via elutriation (Byrd *et al.*, 1976) and centrifugal floatation method (Jenkins, 1964). All nematodes from elutriation and centrifugal floatation method were identified to genus whenever possible under an inverted microscope (Leica DM IL LED, Wetzlar, Germany) and subjected to nematode community analysis. Nematodes counted to the *genus* level were assigned to their trophic groups (bacterivores, fungivores, herbivores, omnivores, and predators) based on categorization of Yeates *et al.* (1993). *Filenchus* and *Tylenchus* were designated as fungivore (Okada and Kadota, 2003). *Prismatolaimus* was classified as a bacterivore instead of substrate digester (Yeates *et al.*, 1993). Taxonomic families were assigned to 1-5 c-p scale (Bongers and Bongers, 1998). Richness was calculated as the total number of taxa per sample. Dominance (λ) was calculated as $\lambda = \sum(p_i)^2$, where p_i is the proportion of each taxon present

(Simpson, 1949) and diversity was calculated as $1/\lambda$. Fungivore (F) to bacterivore (B) ratio was calculated as $F/F+B$ to characterize the dominant decomposition pathways (Freckman and Ettema, 1993). Nutrient enrichment in the soil was indicated by weight abundance of bacterivores with c-p value of one (Ba_1) and fungivores of c-p value of two (Fu_2) calculated as enrichment index (EI) using the equation $100 \times [e/(e+b)]$, in which e is total weight abundance of Ba_1 and Fu_2 and b is the weight abundance of nematodes in the basal food web consisting of Ba_2 and Fu_2 . Characterization of fungal or bacterial decomposition pathway was also represented by channel index (CI), calculated as $100 \times [0.8F_2/e]$ (Ferris *et al.*, 2001). Maturity index (MI) represents nematode fauna weighted by c-p values and calculated as $\sum(v_i f_i)$, in which v_i is the c-p value and f_i is the frequency of the taxon (Bongers, 1990). Resilience, speciousness, and abundance of trophic links associated with the soil food web structure is represented by structure index (SI) calculated as $100 \times [(s/s+b)]$, in which s is the weight abundance of free living nematodes with c-p values higher than 2 (Ferris *et al.*, 2001).

Larvae bait assays: The laboratory larva bait assay (Beeding and Akhurst, 1975) were placed into the petri dishes with 120 g soil (dry weight equivalent) and adjusted to 40% (w/w) soil moisture, prior to exposing 10 mealworm (*Tenebrio molitor*) larva in the soil for 14 days in 140-mm diameter \times 15 mm deep Petri dish for EPN and EPF infections. Mealworm larva were obtained from pet stores. Larva baits were incubated in each dish at room temperature (23 °C), and the mealworm cadavers were examined and collected at 3, 7, and 14 days after the initiation of larvae baiting. Individual insect cadavers detected were surface sterilized in 1% sodium hypochlorite for about 1 minute before rinsing and transferring to individual White Trap made of 6-mm diameter petri dish (White, 1927). White Traps were examined for EPN emergence and

EPF infection approximately 2 weeks after the incubation. Initial EPN and EPF samples were re-inoculated on mealworms to perform Koch's postulate (Kaya and Stock, 1997) analysis. Positive EPN and EPF samples were subjected to molecular identification.

Field cage larva bait assay: Two types of field cages made from 297- μ m or 177- μ m pore screen strainers for spraying equipment (Kleen-rite corp., Columbia, PA) were sealed with lids of FalconTM tubes (ThermoFisher Scientific Inc., Waltham MA) to cover the top and bottom openings of the cage. To get a tight seal, DuckTape[®] (ShurTech brands, Avon, Ohio) was wrapped around the rims of the top and bottom openings to fill the gap between the strainer and FalconTM tube lids slightly modified from McCoy *et al.* (2007). Each field cage contained 5 mealworms larvae close to the final instar and were filled three quarter-full with field soil from treatment plot and buried to about 15-cm deep near the center of each field plot. Each field cage was buried every other week for a total of 7 sampling dates. For Trial I, field cages for the first 3 sampling dates were made of 1,000- μ m pore screen. However, due to loss of mealworm larvae baits from natural predators, 297- μ m pore screen field cage was used thereafter and buried for 7-day exposure period throughout the corn cropping period. In Trial II, 297- μ m pore field cage were buried in the soil for only 2 days to reduce ant foraging rates. In addition, due to ant intrusion into 297- μ m pore field cages, 177- μ m pore field cages were also included to allow for 7-day exposure to EPF infections.

Molecular identification: EPNs collected from White Traps or the EPN IJs extracted from the soil by centrifugal floatation method were randomly selected for species identification using universal nematode primers and sequences. Individual EPNs were cut to rupture the cuticle in 10 μ L of tap water and placed in a 200 μ L PCR tube and prepared for PCR in 35- μ L reaction

(Cabos *et al.*, 2013). Primers were produced by Integrated DNA Technologies (Coralville, IA) targeting ITS region located between 18S and 28S ribosomal genes for the nematode identification. The forward primer sequenced is 5'-TGTAGGTGAACCTGCTGCTGGATC-3', and the reverse primer sequence is 5'-CCTATTTAGTTTCTTTTCCTCCGC-3' (Saeki *et al.*, 2003). EPF samples collected from White Traps were isolated and cultured on potato dextrose agar. An DNeasy® UltraClean Microbial DNA Isolation Kit was used to isolate fungal genomic DNA. Universal fungi primers targeting the ITS region were used for fungi identification. The forward primer of fungi ITS 4 is 5'-GGAAGTAAAAGTCGTAACAAGG-3' and the reverse primer of ITS 4 is 5'-TCCTCCGCTTATTGATATGC-3' (White *et al.*, 1990). Phusion High-fidelity DNA Polymerase (New England Biolabs Inc., Ipswich, MA) was used for polymerase chain reaction (PCR). The conditions for PCR reaction are 98°C for 30 sec followed by 35 cycles of 98°C for 15 sec, 67°C for 15 sec, 72°C for 60 sec, and a final extension for 10 min. Amplified sequences were submitted to Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) at the University of Hawaii at Manoa for DNA sequencing using Sanger sequencing method with Applied Biosystems 3730XL DNA Analyzers (ThermoFisher Scientific Inc., Waltham MA). Sequences were then compared to other sequences in the National Center for Biotechnology Information (NCBI) gene bank with Basic Local Alignment Search Tool (BLAST).

In addition, sequences of the ITS rDNA region of *Heterorhabditis* sp. isolates were used to study the phylogenetic relationships between Hawaii isolate and other known *Heterorahbditis* species. Sequences were aligned by ClustalW in MEGA6, and a phylogenetic analysis was

conducted using the Neighbour-Joining method in MEGA6. *Osheius tipulae* (GeneBank accession no. AJ297895) was selected as the outgroup (Yan *et al.*, 2016).

Soil water properties: Gravimetric soil moistures for each soil sample collected for larvae bait assay were measured by drying the soil in the oven for 48 hours at 70°C. Soil water tension in the corn rhizosphere was monitored using WatchDog Watermark Soil Moisture Sensor (Spectrum Technologies, Inc., Aurora, IL) every hours throughout the corn cropping period. In addition, FieldScout TDR 100 Soil Moisture Meter was used to measure volumetric soil moistures weekly with 12-cm rods at the corn rhizosphere from 3 randomly selected spots per plot. The TDR soil moisture meter was calibrated from taking readings of undisturbed soil cores in which their volumetric soil moisture was determined.

Soil infiltration rate was measured for each plot by single ring infiltration method (Bouwer, 1986) using a 25.4-cm diameter metal ring (infiltrator). Water level inside the infiltrator was maintained at 1 cm for 30 minutes. Steady infiltration rate was derived by the slope of the linear regression line of volume of water infiltrate between 500 to 1800 seconds after initiation of the infiltration test. The infiltration site was then covered with a plastic bag to avoid evaporation and precipitation. Bulk density (Db) was measured 2-days after infiltration by taking a 10-cm diameter polyvinyl chloride (PVC) core to a depth of 10 cm, and then determined by a procedure described by Blake (1965). Soil porosity (Vomocil, 1965) was calculated as $(1 - D_b / D_p)$ assuming 2.85 g/cm^3 as the standard particle density (D_p). Field capacity of the soil was measured from the volumetric soil moisture of the Db core (Peters, 1965). Macroporosity was measured from subtracting volumetric soil moisture at field capacity from total porosity.

Soil temperature: In Trial II, soil temperature probes (WatchDog B-series button data logger, Spectrum® technologies, Aurora, IL) were buried to a 5-cm depth on the day of corn planting in the center of each plot for 12 weeks to record soil temperature hourly.

Thrips assay: Thrips (*Frankliniella occidentalis*) were the target pests of study in these field trials. Yellow sticky traps (13 × 7.5 cm²) were installed in each plot hanging at 30-cm above ground. Sticky traps were left in the field for 1 week at the 6th, 9th, and 12th week of corn growth. The yellow sticky traps were brought back to the laboratory, observed under a dissecting microscope (Leica M125, Buffalo Grove, IL) and counted for numbers of thrips per sticky trap.

Weed management monitoring: Weeds in each plot were treated with glyphosate herbicide on the day of planting and removed manually every 2 weeks after corn planting during the first 4 weeks of critical-weed-free period. Time of manual weeding per person was recorded for each plot as a measure of weed pressure.

Corn growth and yield: Corn height and chlorophyll content were collected monthly from 3 plants per plot. Corn height was measured from the soil level to the top of the plant. Chlorophyll was measured with a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) measuring the third leaf from the top. However, in Trial I, chlorophyll was only taken twice as the final reading at harvest was irrelevant due to early senescence from high infestation of corn leafhopper (*Dalbulus maidis*). Corn cobs were not harvested due to heavy infestation of leafhopper. Instead, shoot biomass of one third the corn plot was weighed. For Trial II, leafhoppers were controlled with insecticide, Sevin® (Novasource, Phoenix, AZ), and a final chlorophyll and cob harvest

was taken. Corn was harvested on the 12 weeks of corn growth from the middle 4 rows from each plot.

Statistical analysis: All data consisting of one sampling date were subjected to one-way analysis of variance (ANOVA) using PROC GLM in SAS 9.3 (SAS Inc, Cary, NC). If normalization of data was required, nematode abundance were log-transformed, $\log_{10}(x+1)$ while all other parameters in percentage or ratio were transformed by $\sqrt{(x + 0.1)}$, before ANOVA. Homogeneity of variance over time was tested for data with multiple sampling dates. If there was no significant interaction between sampling time and treatment effect, data were subjected to repeated measures over times. Means were separated using Waller-Duncan k -ratio ($k=100$) t -test wherever appropriate. Only true mean values were presented. Canonical correspondent analyses (CCA) were used to deduce associations between abundance of IJs of *Heterorhabditis* and *Acrobeloides*, % mortality of mealworms infected by *Heterorhabditis* and *Acrobeloides* from field cages, abundance of nematode in each trophic groups, EI, SI, CI, volumetric soil moisture (SM), volumetric field capacity (FC), soil organic matter (SOM), total soil porosity (TP), macroporosity (MP), water infiltration rate (I), lowest daily soil temperature (LST), highest daily soil temperature (HST), corn yield (Yield), corn height (Height) and corn chlorophyll level (Chl) using CANOCO 4.5 for Windows (Microcomputer Power, Ithaca, NY). *Acrobeloides* parameters were used due to consistent occurrence from larvae bait assays.

2.3 Results

Pre-plant conditions: Black oat biomass fresh weight accumulated at cover crop termination in the no-till BO was 9 tons/ha, while that in Trial II was 36 tons/ha. In Trial I, SOL plots reached a maximum soil temperature of 50.5 °C at 5 cm and 43.5 °C at 15 cm of soil depth, accumulating 301 and 214 hours above the lethal temperature of EPN (which is 37 °C) at 5-cm and 15-cm soil depth, respectively. Whereas BG and BO never accumulated hours above the lethal temperature. In Trial II, since pre-plant treatments were initiated in the cooler months of the year (December to February), SOL plots reached a maximum soil temperature of only 36.0 °C at 5 cm and 33 °C at 15 cm soil depth, and did not accumulate heat above the EPN lethal temperature at both soil depths.

Effects of no-till and black oat cover cropping on soil water and physical properties: No significant interaction was observed between sampling time and treatment effects for both trials. Bulk density (Db) was consistently lighter with more total porosity in SOL, while BO was consistently heavier with less total porosity for both trials ($P \leq 0.05$; Table 2.1). Although Db in BO was equal to BG in Trial I, that in Trial II was higher than BO ($P \leq 0.05$; Table 2.1). As a consequence, BO resulted in lower steady infiltration rate (I) from BG and BO in Trial I ($P \leq 0.05$; Table 2.1), but no difference in I was observed in Trial II ($P > 0.05$). In both trials, macroporosity was consistently lower in BO than BG and SOL ($P \leq 0.05$; Table 2.1), whereas BO maintained the highest field capacity ($P \leq 0.05$). Since no significant interaction was observed between sampling time and treatment effect for weekly volumetric soil moistures, repeated measure analysis over time revealed a consistent trend of $BO > BG \geq SOL$ ($P \leq 0.05$; Table 2.1) in both trials. Soil water tension measured hourly from Watermark probes showed that

BO generally maintaining lower water tension from BG and SOL throughout the corn growth in both trials, especially during periods when water tension was rising 5 weeks after corn planting (Fig. 2.1 A and B).

Soil temperature during the corn growing period was only measured in Trial II at 5-cm soil depth (Table 2.2). BO accumulated less hours of lower temperature for EPN activities in the range of 15-20°C during the morning hours than SOL ($P \leq 0.05$). In addition, BO also accumulated less hours of the higher afternoon temperature range of 30-35 °C ($P \leq 0.05$; Table 2.2) than BG and SOL with a lower maximum temperature of 32°C in BO than the maximum temperature of 36 °C in BG and 37 °C in SOL plots ($P \leq 0.05$, Table 2.2). During the corn growing period, SOL accumulated 4 hours of $\geq 37^\circ\text{C}$ (lethal temperature for EPN, Stuart *et al.*, 2006), while BG and BO did not reach the lethal temperature ($P \leq 0.05$; Table 2.2).

During the corn growing period, 7-day hourly mean soil temperatures were significant different among treatments during the morning and afternoon hours for the first 5 weeks of corn growth (Fig. 2.2). During the early hours of a day, BO maintained warmer soil temperatures than BG and SOL from 2 to 8 am in the first week and 5 to 8 am on the second week ($P \leq 0.05$). During week 3 to 5, BO had higher temperature than SOL at 7 am on the third week, during 6 to 8 am on the fourth week, and during 7 to 8 am on the fifth week ($P \leq 0.05$). On the other hand, in the warmer hours of the day, BO maintained lower temperatures than BG and SOL from 10 am to 5 pm on the first week, and from 11 am to 7 pm on Week 2 and 3 ($P \leq 0.05$). On Week 4, BO was only cooler than SOL from 11 am to 5 pm ($P \leq 0.05$). No significant difference among the treatments during the warmer hours of the day on Week 5 ($P > 0.05$). However, from Week 7 and beyond, BO was consistently warmer than BG and SOL regardless of the hour of the day (P

≤ 0.05), but temperatures did not exceed 29.7 C° in BO with generally cooler soil temperatures than those occurring in weeks 1-6.

Molecular identification: All DNA sequences of EPNs isolated from White Trap before and during Trial I matched 99% to 100% with the unknown species of *Heterorhabditis*. H1 from Johannesburg, South Africa, and SGmg3 from Meghalaya, India (Table 3) reported in National Center for Biotechnology Information (NCBI). A phylogenetic tree (Fig. 3) was derived from 20 published species of *Heterorhabditis* and 3 unpublished species: *Heterorhabditis* sp. H1, *Heterorhabditis* sp. SGmg3, and *Heterorhabditis* sp. SGgi (NCBI, 2017). Fungal DNA sequences from the entomopathogenic fungi isolated from White Trap samples matched 100% with *M. anisopliae* and *B. bassiana* reported in NCBI (Table 2.3).

Effects of no-till and black oat cover cropping on EPN and EPF: Total mortality of mealworms from the laboratory larva bait assay was higher in BO and BG than SOL in Trial I ($P \leq 0.05$) but no difference was detected among treatments in Trial II. Majority of mealworm mortality in the laboratory larva bait assays were associated with *Heterorhabditis*.

Heterorhabditis recovery from the cadavers in laboratory larvae bait assay were greater in BG and BO than SOL consistently in both trials ($P \leq 0.05$; Table 2.4). The next commonly recovered entomopathogens recovered in the larva bait assay was *M. anisopliae*, which was higher in BO than SOL in Trial I ($P \leq 0.05$) and higher in BG and BO than SOL in Trial II ($P \leq 0.05$). The recovery of both *Heterorhabditis* and *M. anisopliae* from the same mealworm cadaver was greater in BO than BG and SOL in Trial II ($P \leq 0.05$; Table 2.4).

Based on the 297- and 177- μm pore field cage larvae bait assay, percent mortality of mealworms associated with *Heterorhabditis* were recovered at higher rates in BO than BG and SOL in both trials ($P \leq 0.05$; Table 2.5; Fig. 2.4). However, effects of soil treatment on the recovery of *Heterorhabditis* from mealworm cadavers from 297- μm pore field cage larvae bait assay varied over time with significant interaction between sampling time and treatment in Trial I ($P \leq 0.05$; Fig. 2.4). Although infection rates of *Heterorhabditis* on mealworm was lower in BO than BG and SOL at Week 2 ($P \leq 0.05$, Fig. 2.4), BO had higher *Heterorhabditis* infection rate than BG and SOL at Week 4 to 8 ($P \leq 0.05$), and had higher *Heterorhabditis* infection rate than SOL from Week 6 to 12 ($P \leq 0.05$) of corn growth. In Trial II, the effect of soil treatment varied over time with significant interaction between sampling time and treatment from the 117- μm pore field cage larvae bait assay. However, infection rates of *Heterorhabditis* on mealworms was greater in BO than BG for week 6 to 10, and greater than SOL for Week 6 and 10 ($P \leq 0.05$).

With the 297- μm pore field cage, total mortalities of mealworm were not different among treatments in both trials ($P > 0.05$, Table 2.5). However, BO had higher mealworm mortality than BG and SOL ($P \leq 0.05$) in the 177- μm pore field cage. *Acrobeloides* recovered from mealworm cadavers in all field cages were consistently highest in SOL in both trials ($P \leq 0.05$).

Effects of no-till black oat cover cropping on soil food web: In Trial I, BO increased F/F+B compared to BG and SOL consistently over the three sampling dates ($P \leq 0.05$). On Week 9, BO had increased abundance of total herbivores, % herbivores and nematode dominance compared to BG ($P \leq 0.05$, Table 2.7). BO was also higher than BG and SOL in CI overtime and % fungivore at Week 0 ($P \leq 0.05$). Although BO did not differ in majority of the soil nematode parameters analyzed compared to the BG in Trial I, abundance of *Heterorhabditis*,

plant-parasitic nematodes including *Helicotylenchus* and *R. reniformis*, and fungivorous nematodes and nematode richness over the three sampling dates during corn growth were lower in SOL than BO ($P \leq 0.05$, Table 2.6). SOL also decreased abundance of *Paratrichodorus* on third sampling date, and bacterivorous and omnivorous nematodes on second sampling date compared to BG ($P \leq 0.05$) in this trial. These had resulted in lower % bacterivores, % herbivores, diversity and MI in SOL than BG on first sampling date ($P \leq 0.05$); lower % fungivores in SOL than BG on the second sampling date ($P \leq 0.05$), but higher dominance of nematode community in SOL than BG in the first and second sampling dates ($P \leq 0.05$, Table 2.7). SOL continued to reduce % fungivores compared to BG on the third sampling date.

In Trial II, BO reduced abundance of *Meloidogyne* and *Pratylenchus* compared to BG, and had higher SI and % omnivore ($P \leq 0.05$) than BG and SOL, but did not affect other nematode community indices compared to BG throughout the corn crop ($P > 0.05$; Table 2.6 and 2.7). SOL reduced abundance of *Meloidogyne*, *Pratylenchus*, bacterivores and fungivores, as well as % fungivores, nematode richness and EI compared to BG control consistently throughout the three sampling dates ($P \leq 0.05$; Table 2.6). SOL also resulted in lower abundance of *Heterorhabditis* in the soil than BG on the first and second sampling dates ($P \leq 0.05$), and lower abundance of bacterivorous nematodes than BG on the second sampling date ($P \leq 0.05$, Table 2.7).

Plant growth, yield and weed pressure: Although corn yields were not different among treatments for both trials ($P > 0.05$, Table 2.8), chlorophyll content was greater in BO than BG in both trials ($P \leq 0.05$). However, plant height was higher in SOL than BO and BG in Trial II ($P \leq 0.05$). This was despite the fact that weed pressure monitored by time of weeding was less in

SOL than BG and BO in both trials ($P \leq 0.05$, Table 2.8), and BO required longer weeding time than BG in Trial II ($P \leq 0.05$).

Species-environment relationship: Relationship between EPNs activities, thrips and abundance of plant-parasitic and free-living nematode trophic groups (species) and soil health indices and all other soil edaphic factors (environment) in Trial I was depicted in an ordination diagram (Fig. 2.5). The first two canonical axes explained 76.4% of the variance in this species–environment multivariate analysis. *Heterorhabditis* abundance is positioned among environmental factors that contribute to the second axis in positive correlation with EI, CI, soil organic matter (SOM), soil moisture (SM), and field capacity (FC), but negatively correlated with infiltration rate (I), total soil porosity (TP) and macroporosity (MP). In addition, abundance of omnivores and bacterivores were in positive correlation with SI and in opposite direction from abundance of fungivores, herbivores, and thrips (*F. willi*). Difference in soil treatments became apparent with the second axis, when sample points were plotted against the canonical axes with SOL positioned in separate direction from BO (Fig. 2.6).

Similar CCA results were obtained for Trial II with addition of *Acrobeloides* abundance and percent larva bait mortality in the field cage larvae bait, but thrips abundance was deleted from the species variables due to insignificant counts with an average of less than 3 per trap (97 cm²). On the other hand, plant growth parameters (chlorophyll content, plant height), lowest daily soil temperature (LST), and highest daily soil temperature (HST) were added to the environmental variables. The first two canonical axes explained 76.9% of the variation in this species–environment multivariate analysis and was depicted in Fig. 2.7. *Heterorhabditis* abundance and infection rates from field cage larva bait assays (H297 and H177) were positively correlated with

SOM, SM, FC, LST, SI, EI, abundance of omnivores, height, and yield, while *Acrobeloides* abundance and occurrence from field cage (A297 and A177) were positively correlated with TP, MP, I, HST and CI. Difference in treatments become apparent with the first axis when sample points are plotted against the canonical axes with SOL positioned in separate direction from BO (Fig. 2.8).

2.4 Discussion

No-till cover cropping improve soil physical properties: No-till has commonly been reported to result in greater soil compactness with higher bulk density in farmland, forest, and grazing pastures compared to conventional till system (Alvarez and Steinbach, 2009; Dam *et al.*, 2004; Gantzer and Blake, 1978; Hill, 1990; Reichert, 2009; Suzuki *et al.*, 2013; Vyn and Raimbault, 1993; Wander *et al.*, 1998) especially in a long-term no-till field (Horn, 2004; Reichert *et al.*, 2016). However, there are reports of no-till reducing bulk density in loamy Ultisols (Franzluebbers, 2001; Lal *et al.*, 1994). The Haplustox in our experiments revealed that 8 years of no-till resulted in more compacted soil with higher bulk density and lower total porosity which has been commonly reported for Oxisols and Ultisols (Hill, 1990; Reichert *et al.*, 2009; Reichert *et al.*, 2016; Tormena *et al.*, 1999). However, soil water retention was improved by no-till practice as field capacity and volumetric soil moistures were higher in BO than BG, consistent with that reported in a 14 year no-till Hapludox in Brazil (Reichert *et al.*, 2016), 6 year no-till Argiudoll (Gantzer and Blake, 1978), and an Argiudolls of southern pampas of Argentina (Fabrizzi *et al.*, 2005). This could be due to the fact that tillage increases macroporosity, but does

not preserve microporosity and capillary pores (Arshad *and* Azooz, 1995) responsible for water holding capacity. Hill (1990) also reported that conventional tillage retained more moistures at low water potentials (0-2 kPa) in its macropores, while no-till retained more moistures at higher water potentials (3.9 to 40 kPa) in a Hapludults from Maryland. A similar result was observed by Tormena *et al.* (1999) in Hapludox in Brazil, and by Arshad and Azooz (1995) in a Canadian Cryoboralfs. During periods of water stress (soil water tensions reached the range of 3.9 to 40 kPa) of the current experiment, BO maintained lower water potentials than BG and SOL, making soil water more available for plants to uptake. Higher soil moisture in BO could be the result of more micropores as reflected in its higher field capacity. Organic mulch from the no-till black oat cover crop may have also helped to reflect solar radiation (Arshad and Azooz, 1996), maintain cooler temperatures (Fabrizzi *et al.*, 2005), thus reduce soil evaporation and maintain higher soil moistures. All of these improvement in edaphic factors are believed to contribute to higher abundance and infectivity of *Heterorhabditis* in BO.

Increased in soil organic matter in the no-till BO plot is another factor in conserving soil moisture. SOM was generally below 2% for tropical Oxisols (Calegari *et al.*, 2008). No-till practice generate surface organic mulch (Hunt *et al.*, 1996; Lal, 2009) and the aggregation of soil organic matter with variable-charge from iron and aluminum oxides helps to form microaggregates (< 250 μm) (Calegari *et al.*, 2008; Beare *et al.*, 1994) especially in no-till Oxisols (Zotarelli *et al.*, 2007). Unfortunately, higher SOM is also known to enhance predators and antagonistic organisms against EPNs (Klingen and Haukeland, 2006).

Despite higher percent of SOM, water infiltration rate was slower in BO than SOL, though not different from BG. Higher SOM is usually expected to improve soil aggregation and

porosity, but lower total porosity and macroporosity in no-till have been reported to reduce saturated hydraulic conductivity (Reichert *et al.*, 2016; Gantzer and Blake, 1978; Hill *et al.*, 1985; Guzha, 2004), similar to the results obtained in this study.

Effects of no-till cover cropping on EPNs and EPF: The most abundant EPNs found in this field site were *Heterorhabditis* sp. SGmg3 and H1, consistent with a recent EPNs surveys in Hawaii (Meyers *et al.*, 2015). This unclassified indigenous *Heterorhabditis* species were detected more frequently from laboratory larva bait assay and field cage larva bait assays than other free-living bacterivorous nematode such as *Acrobeloides* spp. This *Heterorhabditis* had also been re-infected and re-isolated on mealworms to confirm their pathogenicity.

No-till cover cropping of BO did not enhance occurrence of EPF but solarization did reduce their occurrence. The most frequently detected EPF from laboratory larvae bait assay were *M. anisopliae* and occasionally *B. bassiana* from the 177- μ m pore field cage larvae bait assay only in Trial II. DNA sequence analysis of *M. anisopliae* isolated matched an indigenous strain, Hawaii Five-O strain, isolated on Oahu (NCBI, 2017) which is adapted to the sub-tropical climate. However, detection of *M. anisopliae* was sporadic and only occurring in the 177- μ m pore field cages in Trial II possibly from longer exposure in the field (7 days) compared to the short exposure period (2 days) of 297- μ m pore field cages. Laboratory larvae bait assay was a better detection method for EPF as soil samples were composited from 6 soil cores per plot, moisture was adjusted to 40% (wt/wt), and the larva baits were incubated for 2 weeks.

In contrast, field cage larva bait assay is a more effective detection method for *Heterorhabditis*. For example, laboratory larva bait assay only detected 30% infectivity of

Heterorhabditis but the field cage assay with 297 or 177- μm pores detected 55% and 52.5%, respectively. While the laboratory larvae bait assay did not detect difference in *Heterorhabditis* infectivity between BO and BG, the field cage took advantage of the migratory foraging behavior of *Heterorhabditis*, allowed the improved soil conditions in no-till cover cropping with BO to increase infectivity of mealworms by *Heterorhabditis* compared to conventional tillage in BG and solarization. Soil conditions in BO may have improved migratory distance of EPNs, as was observed with *Steinernema carpocapsae* which forages by ambushing and migrated at a rate up to 33.3 cm/day in an agroecosystem with mix dicot-monocot cover crops as ground cover to increase soil moistures (Jabbour and Barbercheck, 2008). Higher soil moistures in BO than BG and SOL could have improved migration of IJs of EPNs by providing a water film on soil particles (Wallace, 1971; Barbercheck, 1992), and enhanced EPN infectivity and virulence (Grant and Villani, 2003; Jabbour and Barbercheck, 2008; Klein, 1990; Shapiro-Llan *et al.*, 2002).

Soil conditions in BO may have also improved infectivity of *Heterorhabditis* by maintaining lower temperatures during the midday hours, while BG and SOL held temperatures at or near the lethal temperature for EPN of 37°C (Stuart *et al.*, 2006). High soil temperatures may cause IJs to migrate deeper into soil profile to cooler temperatures, resulting in less IJs at corn rhizosphere (10 cm depth) where the field cage larva baits were installed. Higher occurrence of *Acrobeloides* and concomitant infections of *Heterorhabditis* on the larva baits in BG and SOL than BO suggested that *Acrobeloides* is an opportunistic bacterivores, and have been reported to interfere with EPN infections (Campos-Herrera *et al.*, 2012; Campos-Herrera *et al.*, 2013; Duncan *et al.*, 2003; Jaffuel *et al.*, 2016).

Though there was no significant difference of *M. anisopliae* infections between BG and BO, concomitant infections of *Heterorhabditis* and *M. anisopliae* were higher in BO though at a relatively low rate (9.2%) than BG from the laboratory larvae bait assay. This suggested that these indigenous strains of EPN and EPF may have adapted to coexist and not outcompete with each other under BO no-till conditions.

Effects of no-till cover cropping on soil health: In general, BO treatment in Trial I was dominated by fungal decomposition pathways as suggested by higher F/(F+B) and CI. No-till is known to have less bacterivores and more fungivores than conventional tillage (Hendrix *et al.*, 1986). This is expected when the cover crop residues have high carbon to nitrogen ratio (Ferris and Matute, 2003) of non-labile carbon sources that favor fungal decomposition. Zhang *et al.* (2012) also reported that no-till resulted in higher CI than conventional tillage, but would increase EI and SI if biomass of cover crop residues increases. Four-fold increase in black oat residues in BO of Trial II compared to Trial I did increase % omnivores and SI, and reduced CI compared to the BG control. A higher structured soil food web in BO than other treatments of Trial II also suggested that more than one cropping cycle was needed to improve the soil food web with BO.

Four-fold increase in black oat biomass in Trial II than Trial I also led to a more structured soil food web and better suppression of plant-parasitic nematodes, similar to that reported by LaMondia *et al.* (2002). *Meloidogyne* and *Pratylenchus* was greater in BG than BO and SOL. This result could also be due to BO is not a host to *Meloidogyne* (Lima *et al.*, 2009) and possibly *Pratylenchus* (LaMondia *et al.*, 2002), while BG had weeds growing during the fallow period.

High soil temperatures from solarization was effective in reducing herbivores for at least 9 weeks after corn planting in Trial I. However, suppression of herbivores in SOL did not last till the last sampling date of the corn. Solarization did not reach the lethal temperature for nematodes in Trial II due to overcast weather and more frequent precipitation during the solarization period. Therefore, in Trial II, % herbivore was greater in SOL than BG and BO. None-the-less, SOL consistently disrupted the nematode community and exhibited a soil lower in abundance of bacterivores, fungivores, omnivores, richness, EI and SI than BG and BO in both trials.

Relationship between EPN and soil conditions: Canonical correspondence analysis in both trials suggested that abundance of *Heterorhabditis* was positively correlated with volumetric soil moisture, soil organic matter and field capacity. Soil moisture has been known to be one of the most important factors affecting the movement, infectivity, and virulence of EPN (Shapiro-Llan *et al.*, 2002; Klein, 1990). However, infiltration rate and soil porosity were negatively correlated with *Heterorhabditis* abundance. This is due to the fact that increase soil infiltration and porosity is associated with dryer soil. Soil tillage increased macropores and total porosity but at the cost of reducing micropores (Arshad and Azooz, 1995) and the ability of soil to retain moistures at higher water tensions, i.e. drier conditions (Hill, 1990), all of which are unfavorable for EPNs.

Corn thrips, *F. williamsi*, is an insect pest on corn that vector an important corn virus in Hawaii, *Maize chlorotic mottling virus* (MCMV) (Jiang *et al.*, 1992). This insect shares part of their life cycle in the soil during pupation at the depth of 1.5 to 2 cm consisting of one third their lifecycle and are therefore a host to EPNs (Ebssa *et al.*, 2001). Thus thrips is a potential host of the indigenous *Heterorhabditis* in this study. Though not statistically significant in ANOVA, a negative relationship between abundance of *Heterorhabditis* and thrips was apparent in the

ordination diagram of Trial I. However, this could not be replicated in Trial II due to low detection level of *F. williamsi*.

Abundance of *Heterorhabditis* was also correlated with many other soil health indicators based on nematode community analysis. Consistent positive correlation between *Heterorhabditis* abundance with EI could be associated with nutrient enrichment from the cover crop residues and higher biodiversity and abundance of alternative arthropods hosts associated with no-till cover cropping system. Previous studies at the same experiment site with no-till cover cropping of sunn hemp (*Crotalaria juncea*) increased abundance and diversity of beneficial soil dwelling arthropods on the soil surface, and lead to significant reduction of *F. occidentalis* damage on onion (Quintanilla-Tornel, *et al.*, 2016). In Trial I, there was no correlation of *Heterorhabditis* abundance with SI since the BO did not generate sufficient biomass to improve SI. However, in Trial II when BO biomass was four times greater than Trial I, SI was highly correlated with *Heterorhabditis* abundance and infectivity in 177- μm pore.

On the other hand, infectivity of *Heterorhabditis* based on results from both field cage larva bait assays was positively correlated with either corn height, chlorophyll content, and yield. Tritrophic interactions among insect pests, EPNs and target crops have been known to play a role in affecting infectivities of EPNs (Hiltpold *et al.*, 2010 a, b). One hypothesis proposed is that insect herbivores could induce a plant volatile, herbivore induced plant volatiles (HIPVs), such as (*E*)- β -caryophyllene released from corn that have been known to attract EPN when herbivorous insects feed on the plant (Chiriboga *et al.*, 2017; Hiltpold *et al.*, 2010 a, b; Rasmann *et al.*, 2005). However, higher insect pressure may be needed for effective recruitment of EPN by HIPV synthesis.

A positive correlation between *Acrobeloides* found in larva baits from the field cage and various edaphic factors (infiltration rate, macroporosity, total porosity, and highest daily soil temperatures) and CI unfavorable for *Heterorhabditis* suggested that *Acrobeloides* are behaving like an opportunistic scavenger. *Acrobeloides* could concomitantly infect an insect cadaver infected by *Heterorhabditis* and consume insect nutrients when conditions are harsh for EPNs because *Acrobeloides* is known to be a nematode with basal guild, having the competitive advantage in unfavorable conditions (Ferris et al., 2001). In general, the apparent separation of the scattered plot of species variables of BO and SOL in both trials on the canonical axes further confirmed that no-till cover cropping with black oats can improve soil health conditions favorable for *Heterorhabditis* infectivity and abundance.

2.5 Conclusion

Though *Heterorhabditis* abundance was not increased by BO consistently, this study provided comprehensive evidences that no-till cover cropping with black oats can improve soil health conditions and edaphic factors favorable for the infectivity of indigenous *Heterorhabditis* in Hawaii and possibly other EPNs. However, no-till cover cropping with black oat did not improve soil conditions for *M. anisopliae*. This study also revealed that the occurrence of *Acrobeloides* from mealworm cadavers in the field cage larva bait assay was a good indicator of an unfavorable environment for *Heterorhabditis*. Canonical correspondence analysis revealed that a tritrophic interaction among EPNs, insect host and the corn exist. This warrants further investigation to reduce thrips population in corn agroecosystem below the economic threshold through conservation agricultural practices.

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Table 2.1. Effects of tillage practices on soil physical properties in corn agroecosystem from field plots of no-till black oat (BO), bare ground (BG), and solarization (SOL) plots.

Soil properties	Trial I			Trial II		
	BG	BO	SOL	BG	BO	SOL
Db ^y (g/cm ³)	1.01 ^z A	1.06 A	0.95 B	1.1 B	1.2 A	1.0 B
I (cm/hour)	42.2 B	22.3 B	135.7 A	33.2 A	18.8 A	27.9 A
FC (θ _v)	32.5 B	38.5 A	30.6 B	34.8 B	37.4 A	32.6 C
TP (%)	64.6 B	62.9 B	66.7 A	62.1 A	59.4 B	63.1 A
MP (%)	32.1 A	24.3 B	36.1 A	27.4 B	22.0 C	30.5 A
SOM (%)	1.08 B	1.45 A	0.98 C	1.1 B	1.5 A	0.9 B
SM (θ _v)	33.0 B	39.6 A	28.5 C	32.4 B	36.9 A	32.4 B

^z Means are average of 4 replications in repeated measures of planting and harvesting for Db, I, FC, TP, MP, and SOM (n = 8), and weekly samples for SM in Trial I (n = 44) and Trial II (n = 48). Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

^y Db = bulk density, I = Steady infiltration rate, FC = Volumetric field capacity, TP = Total porosity, MP = macroporosity, SOM = Soil organic matter, and SM = Average volumetric soil moisture throughout the corn growing period.

Table 2.2. Effects of tillage practices on total hours of soil temperature ranges in corn agroecosystem from field sites of no-till black oat (BO), bare ground (BG), and solarization (SOL) plots of Trial II.

Temperature (°C)	BG	BO	SOL
	Total hours		
15-20	144 ^z AB	78 B	183 A
30-35	92 A	17 B	111 A
35-37	2 B	0 B	14 A
>=37	0 B	0 B	4 A
Max ^y	36 A	32 B	37 A
Min	17 A	18 A	17 A

^z Mean of total hours within a temperature range. Means are average of 4 replications. Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

^y Max = mean maximum temperature reached throughout the corn growing period, Min = mean minimum temperature reached throughout the corn growing period.

Table 2.3. Molecular identification of entomopathogenic nematode and fungi isolated from field sites of no-till black oat (BO), bare ground (BG), and solarization (SOL) plots of Trial I.

Isolate	Molecular identification (accession number for GeneBank match)
BO1-9	99% match to <i>Heterorhabditis sp. SGmg3</i> (FJ751864) and H1 (KM387406)
BO1-15	99% match to <i>Heterorhabditis sp. SGmg3</i> (FJ751864) and H1 (KM387406)
BO1-18	99% match to <i>Heterorhabditis sp. SGmg3</i> (FJ751864) and H1 (KM387406)
BO1-21	99% match to <i>Heterorhabditis sp. SGmg3</i> (FJ751864) and H1 (KM387406)
BO1-24	99% match to <i>Heterorhabditis sp.</i> H1 (KM387406)
BO1-30	99% match to <i>Heterorhabditis sp.</i> H1 (KM387406)
BO1-31	100% match to <i>Beauveria bassiana</i> strain 88 (HM189220)
Q1 ^z -12	99% match to <i>Heterorhabditis sp.</i> H1 (KM387406)
Q1-6	97% match to <i>Heterorhabditis sp.</i> H1 (KM387406)
T-7	100% match to <i>Metarhizium anisopliae</i> clone Hawaii Five-O (KT992823)

^z Isolates labeled with T and Q1 are from preliminary samples collected prior to initiation of the experiments; isolates labeled with BO1 were from Trial I during corn growth. All DNA samples of entomopathogenic fungi or nematodes were isolated from White Traps or infective juveniles from soil extraction.

Table 2.4. Effects of no-till cover cropping with black oats (BO) compared to bare ground from conventional tillage (BG) and solarization (SOL) on percent of *Tenebrio molitor* larvae infected by nematodes and/or fungi from laboratory larva bait assays.

Organisms recovered	Trial I			Trial II		
	BG	BO	SOL	BG	BO	SOL
<i>Acrobeloides</i>	3.3 ^z A	3.3 A	5.0 A	12.5 A	8.3 A	18.3 A
<i>Heterorhabditis</i>	18.3 A	20.0 A	2.5 B	21.7 AB	33.3 A	11.7 B
<i>Metarhizium</i>	13.3 AB	15.8 A	0.0 B	35.8 A	34.2 A	15.0 B
HA ^y	0.0 A	3.3 A	0.0 A	1.7 A	0.0 A	1.7 A
MA	-	-	-	3.3 A	0.8 A	1.7 A
MH	-	-	-	2.5 B	9.2 A	0.8 B

^z Means are average of 4 replications. Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

^y HA = *Heterorhabditis* and *Acrobeloides*, MA = *Metarhizium* and *Acrobeloides*, MH = *Metarhizium* and *Heterorhabditis*

Table 2.5. Effects of no-till cover cropping with black oats (BO) compared to bare ground from conventional tillage (BG) and solarization (SOL) on percent of *Tenebrio molitor* larvae infected by different nematodes and/or fungi in field cages with 297- μm or 177- μm pore screens buried in soil.

Sieve pore size (μm)	Organisms recovered	Trial I			Trial II		
		BG	BO	SOL	BG	BO	SOL
297	<i>Acrobeloides</i>	5.0 ^z B	1.4 B	13.2 A	6.4 B	0.0 C	12.1 A
	<i>Heterorhabditis</i>	22.6 B	42.4A	8.3 C	7.9 B	55.0 A	13.6 B
	HA ^y	0.0 A	0.7 A	0.0 A	0.0 B	0.0 B	2.9 A
177	<i>Acrobeloides</i>	-	-	-	2.1 B	0.7 B	17.1 A
	<i>Heterorhabditis</i>	-	-	-	26.4 B	52.5 A	17.1 B
	<i>Metarhizium</i>	-	-	-	2.1 A	1.4 A	0.7 A
	HA	-	-	-	0.0 B	0.0 B	4.3 A
	MH	-	-	-	0.7 A	2.1 A	0.0 A

^z Means are average of 4 replications. Means in a row followed by the same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t -test.

^y HA = *Heterorhabditis* and *Acrobeloides*, MH = *Metarhizium* and *Heterorhabditis*

Table 2.6. Effect of no-till cover cropping with black oat (BO) on nematode communities compared to bare ground (BG) and soil solarization (SOL) during corn growing season.

	Trial I			Trial II		
	BG	BO	SOL	BG	BO	SOL
<u>Nematode abundance</u>	Nematode / 250cm ³ soil					
<i>Heterorhabditis</i> ^z	10 ^z AB	13 A	0 B	93 B	133 A	0 C
<i>Helicotylenchus</i>	3 AB	21 A	0 B	13 AB	16 A	0 B
<i>Meloidogyne</i>	10 A	3 A	137 A	133 A	76 B	336 B
<i>Paratrichodorus</i>	0 A	0 A	0 A	15 A	2 A	22 A
<i>Pratylenchus</i>	0 A	0 A	0 A	166 A	13 B	10 B
<i>Rotylenchulus</i>	152 A	288 A	48 B	611 A	464 A	627 A
Bacterivore	373 A	178 B	151 C	659 A	542 A	293 B
Fungivore	156 A	201 A	17 B	307 A	255 A	131 B
Herbivore	170 A	313 A	185 B	938 A	571 A	997 A
Omnivore	10 A	8 A	1 A	31 AB	49 A	17 B
Predator	2 A	1 A	0 A	3 A	4 A	1 A
<u>Nematode indices</u>						
% Bacterivore	44.3 A	25.7 B	39.8 B	32.6 A	35.3 AB	27.4 B
% Fungivore	22.3 B	29.1 A	3.6 C	15.9 A	17.0 A	10.7 B
% Herbivore	28.0 AB	42.6 A	22.8 B	48.6 AB	42.4 B	60.1 A
% Omnivore	1.3 A	1.0 A	0.1 A	1.7 B	3.3 A	1.1 B
% Predator	0.3 A	0.1 A	0.0 A	0.2 A	0.3 A	0.0 A
Diversity	8.1 A	5.1 AB	4.4 B	7.0 A	6.8 A	11.7 A
Dominance	16.5 A	23.0 A	18.4 A	20.7 B	19.9 B	32.9 A
Richness	13 A	12 A	4 B	17 A	17 A	10 B
CI ^y	31.8 B	55.8 A	20.8 B	36.4 A	36.9 A	58.3 A
EI	57.2 A	52.9 A	9.0 B	56.7 A	59.6 A	34.2 B
F/F+B	33.3 B	53.4 A	4.0 C	34.7 A	31.8 A	27.8 A
MI	1.9 B	2.0 A	1.3 C	1.9 A	2.0 A	2.0 A
SI	11.9 A	11.6 A	2.6 B	20.4 B	38.8 A	13.1 B

^z Means are repeated measures over 3 dates from 4 replications (n = 12). Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test based on log-transformation, log(x+1), for nematode abundance, or square root transformation, $\sqrt{(x + 0.1)}$, for other parameters wherever necessary prior to ANOVA.

^y CI = Chanel index, EI = Enrichment index, F/F+B = ratio of the abundance of fungivores to bacterivores, MI = maturity index, and SI = Structure index.

Table 2.7. Effects of pre-plant soil treatments of black oat (BO) in no-till nematode community compared to bare ground (BG) and soil solarization (SOL) in a corn agroecosystem separated by date.

Nematode parameters	Week	Trial I			Trial II		
		BG	BO	SOL	BG	BO	SOL
<i>Heterorhabditis</i> ^z	0	-	-	-	18 A	20 A	0 A
	9	-	-	-	248 A	350 A	0 B
	12	-	-	-	30 AB	28 A	0 B
<i>Paratrichodorus</i>	0	0 ^z A	0 A	0 A	-	-	-
	9	0 A	0 A	0 A	-	-	-
	12	13 A	3 B	0 B	-	-	-
Bacterivore	0	105 A	135 A	0 A	-	-	-
	9	330 A	128 B	125 B	-	-	-
	12	683 A	273 A	328 A	-	-	-
Herbivore	0	113 A	58 A	0 B	-	-	-
	9	145 B	338 A	28 B	-	-	-
	12	253 A	443 A	528 A	-	-	-
Omnivore	0	0 A	3 A	0 A	-	-	-
	9	23 A	0 B	0 B	-	-	-
	12	8 A	23 A	3 A	-	-	-
% Bacterivore	0	33.8 A	25.8 AB	0.0 B	34.5 A	25.0 AB	14.8 B
	9	47.8 AB	22.0 B	72.0 A	40.0 A	50.8 A	29.3 A
	12	51.5 A	29.3 A	47.3 A	23.0 A	30.0 A	38.3 A
% Fungivore	0	20.0 B	40.5 A	0.0 C	-	-	-
	9	23.8 A	25.0 A	4.5 B	-	-	-
	12	23.0 A	21.8 A	6.3 B	-	-	-
% Herbivore	0	41.0 A	32.5 A	0.0 B	-	-	-
	9	20.5 B	50.8 A	23.5 B	-	-	-
	12	22.5 A	44.5 A	44.8 A	-	-	-

Table 2.7 continued

Nematode parameters	Week	Trial I			Trial II		
		BG	BO	SOL	BG	BO	SOL
Diversity	0	6.2 A	5.6 A	0.0 B	-	-	-
	9	10.2 A	3.9 A	3.1 A	-	-	-
	12	8.1 A	5.7 A	10.0 A	-	-	-
Dominance	0	25.0 A	12.0 B	12.5 A	26.8 B	36.3 AB	59.0 A
	9	18.5 AB	28.8 A	21.8 A	18.3 A	13.0 A	31.3 A
	12	0.0 B	35.0 A	20.3 A	17.0 A	10.5 A	8.5 A
MI	0	1.8 A	1.9 A	0.0 B	-	-	-
	9	2.0 A	1.9 A	2.0 A	-	-	-
	12	1.9 A	2.0 A	2.0 A	-	-	-

^z Means are average of 4 replications. Means in a column followed by the same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t -test.

^y MI = maturity index, SI = structure index

Table 2.8. Effect of no-till cover cropping with black oat (*Avena stringosa*) on corn growth, yield and weed pressure.

Growth parameters	Trial I			Trial II		
	BG	BO	SOL	BG	BO	SOL
Yield ^y (kg/ha)	†8230 ^z A	8538 A	8648 A	6331 A	6815 A	6001 A
Chlorophyll (SPAD)	32.7 B	38.2 A	37.2 A	41.6 B	45.1 A	43.4 AB
Height (cm)	100.4 A	97.2 A	100.6 A	146.0 B	145.0 B	162.0 A
TW (Sec/Person)	379.0 A	403.5 A	75.5 B	87.9 B	116.4 A	37.0 C

^z Means are average of 4 replications. Means in a row followed by the same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t -test.

^y Height and chlorophyll consist of 3 repeated measures, smut data collected at harvest, TW = time of weeding repeated measure twice per trial.

†Biomass was record from trial I instead of yield due to lack of harvestable cobs.

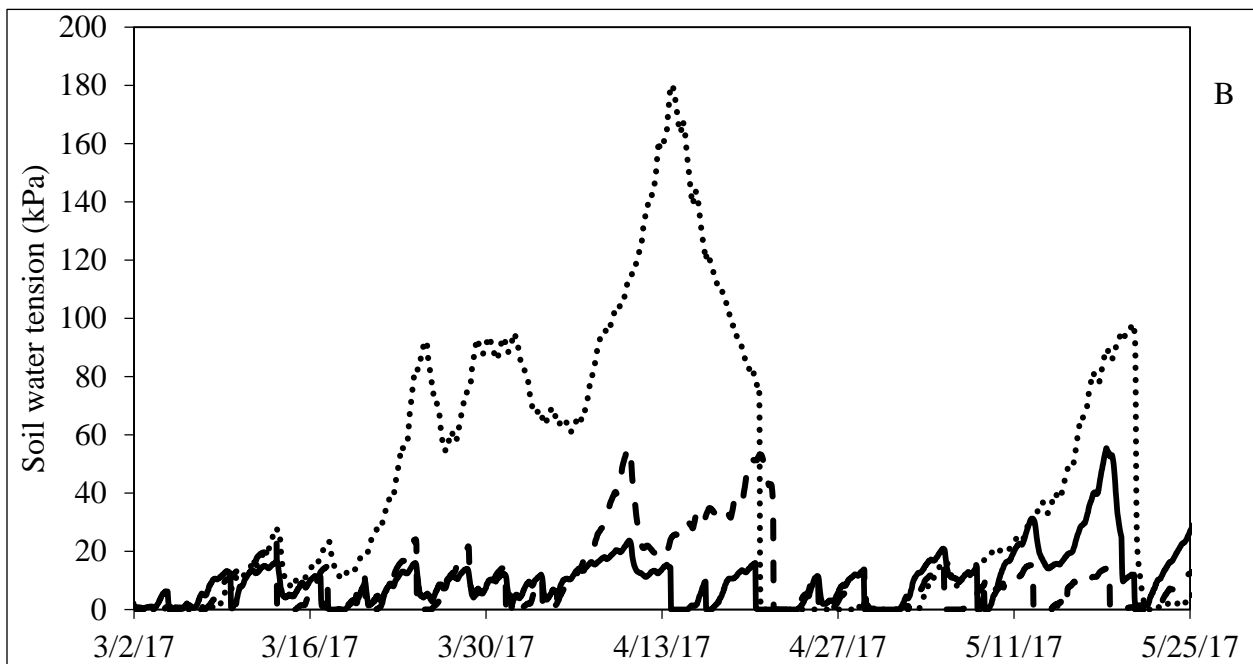
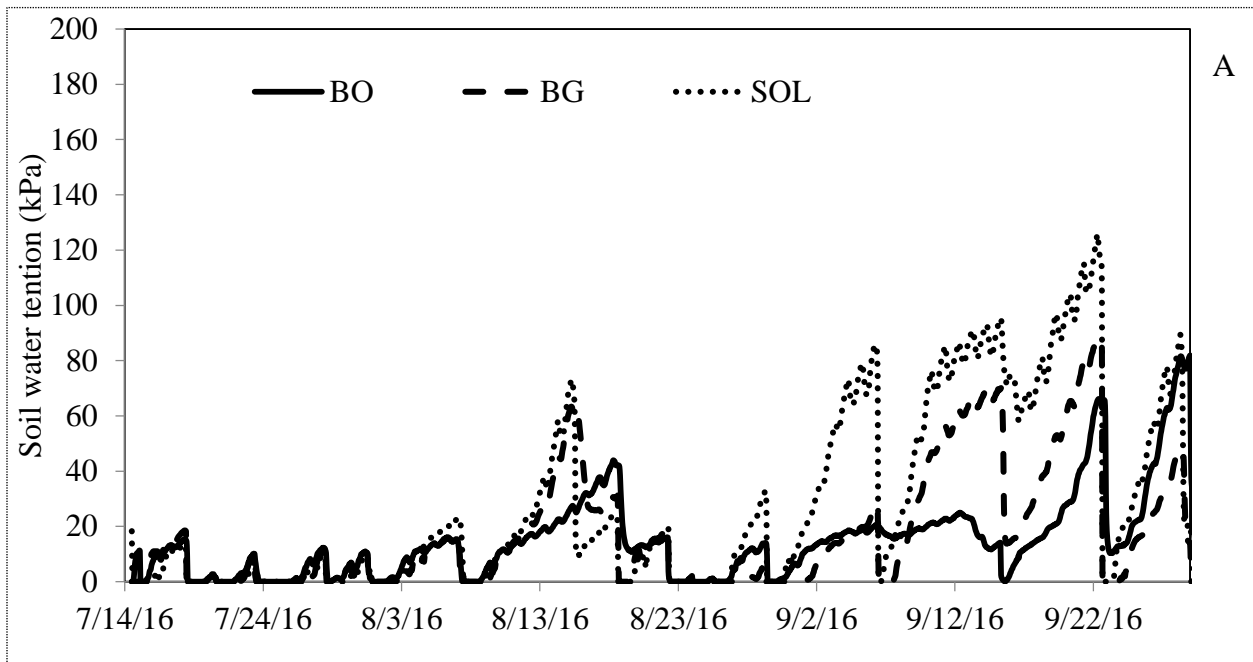


Fig. 2.1. Soil water tension recorded hourly throughout the 3-month corn growing period in A) Trial I and B) Trail II: BG = bare ground conventional tillage, BO = no-till with black oat cover crop, SOL = solarized.

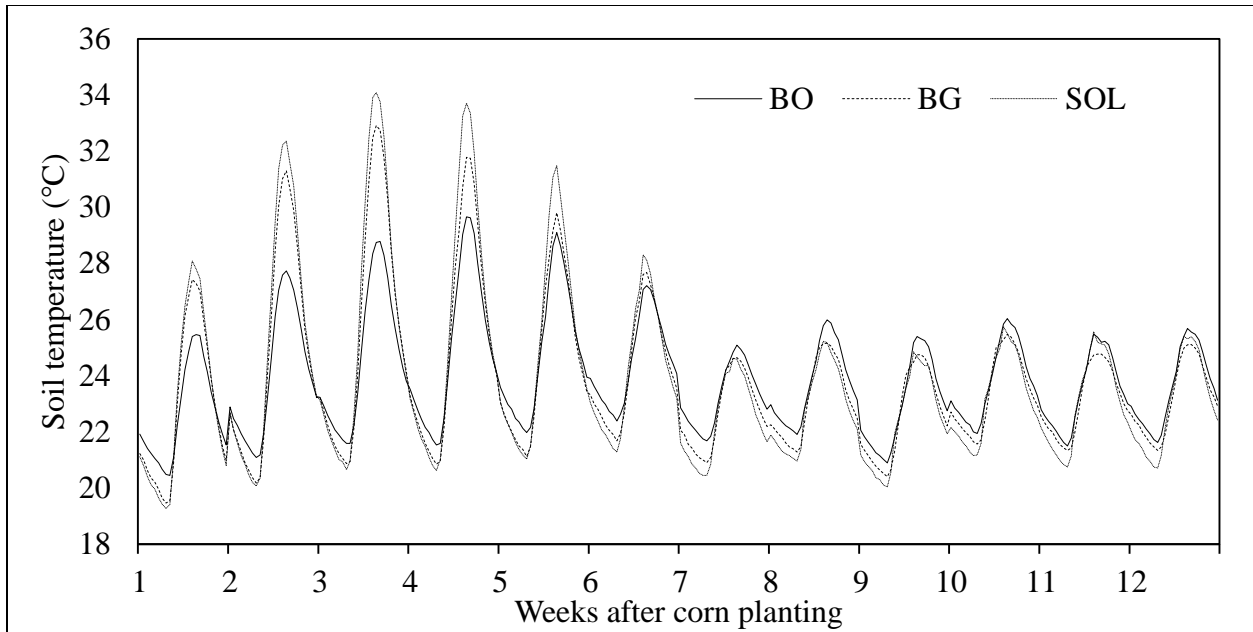


Fig. 2.2. Effect of organic mulch from black oat plant residue on no-till (BO) compared to bare ground from conventional tillage (BG) and solarization (SOL). Means of 7-day soil temperature recorded hourly throughout the 3 months of corn growing period in Trial II: BG = bare ground conventional tillage, BO = no-till with black oat cover crop, SOL = solarized.

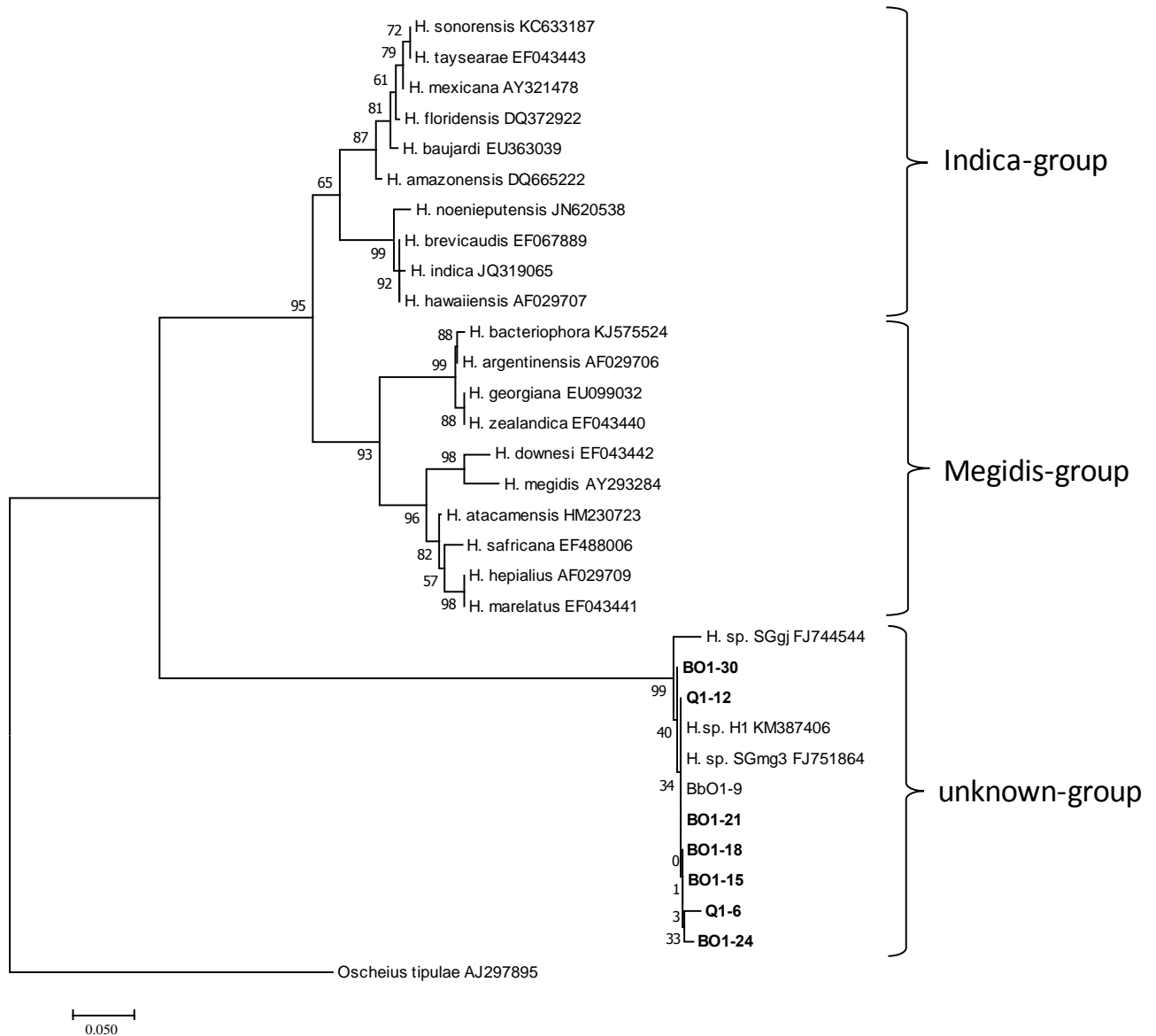


Fig. 2.3. Phylogenetic tree inferred from neighbor-joining tree method for internal transcribed spacer (ITS) of rRNA gene sequences from *Heterorhabditis* spp. with *Oscheius tipulae* as an outgroup. Isolates in the bold font are isolated from Trial I at the Poamoho Experiment Station, Oahu, HI. Bootstrap values are shown as % of 1000 replications. Lengths of branches are drawn to the scale of evolutionary distance units.

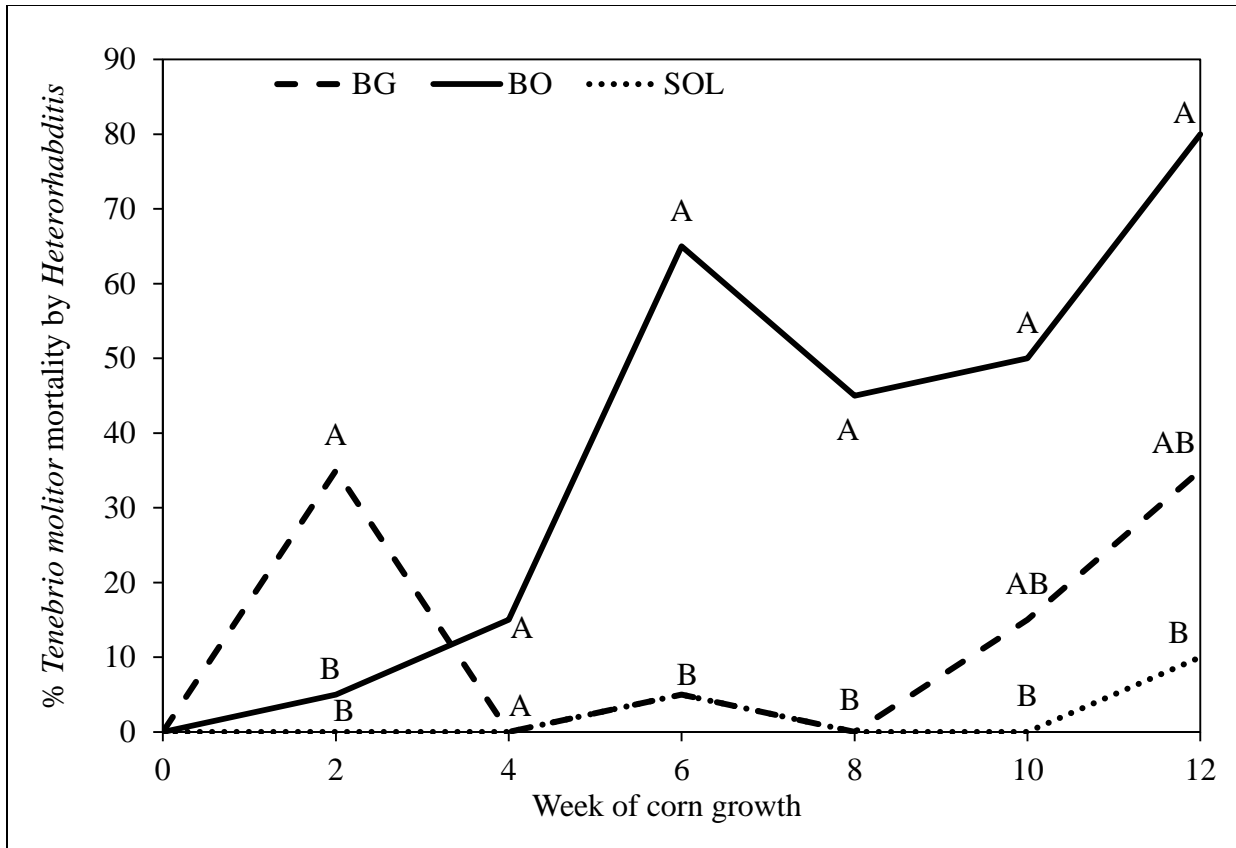


Fig. 2.4. Effect of no-till cover cropping with black oats (BO) compared bare ground by conventional tillage (BG) and solarization (SOL) on *Heterorhabditis* occurrence from field cages of mealworm (*Tenebrio molitor*) larvae baits. Biweekly means of percent of *Tenebrio molitor* infections by *Heterorhabditis* from field cage larvae bait assay throughout the corn growing period in Trial I: BG = bare ground conventional tillage, BO = no-till with black oat cover crop, SOL = solarized.

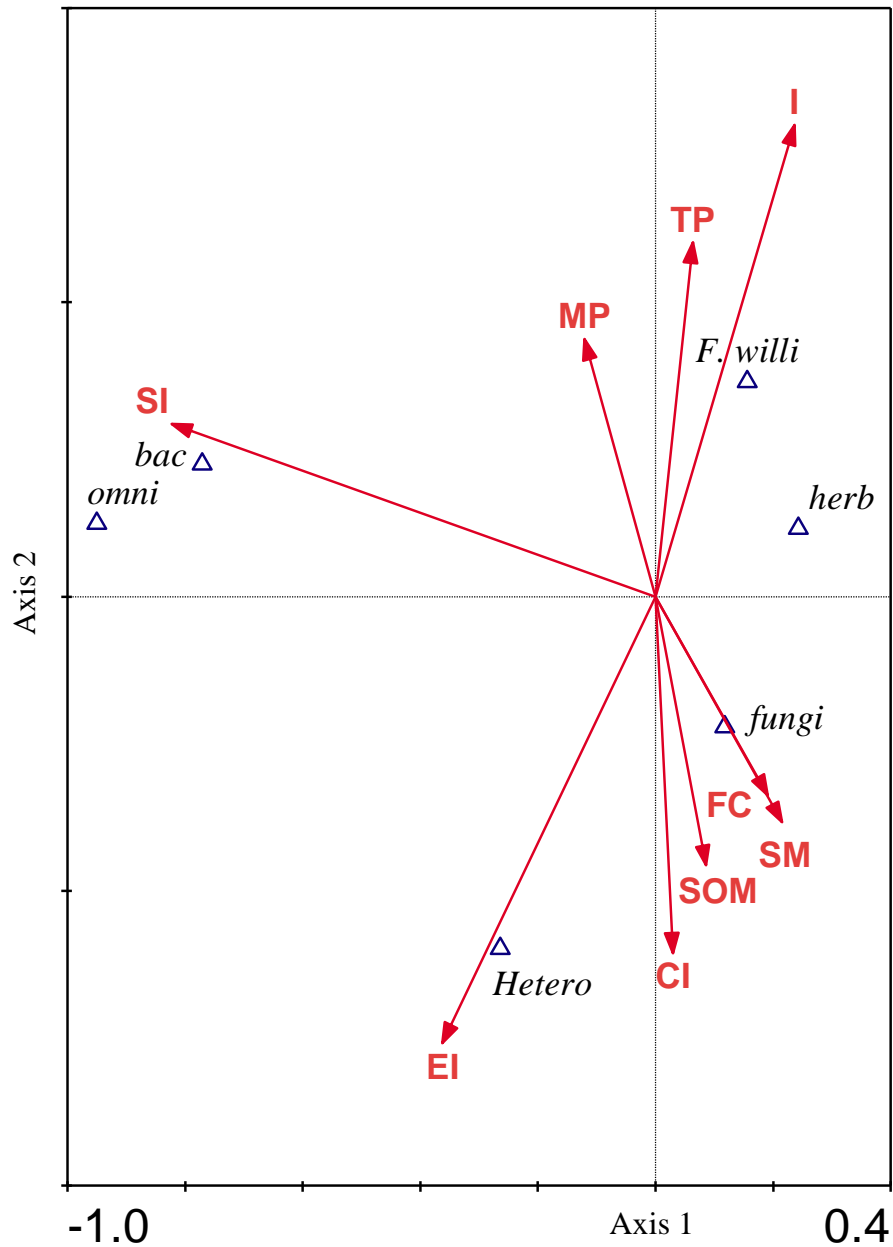


Fig. 2.5. Ordinance diagram depicts the first two canonical axes of a canonical correspondence analysis between species variables (abundance of *Heterorhabditis*, *Hetero*; nematode trophic groups including bacterivores, bac; fungivores, fungi; herbivores, herb; and omnivores, omni; and thrips, *F. willi*), and environmental variables (channel index, CI; enrichment index, EI; structure index, SI; field capacity, FC; infiltration, I; total porosity, TP; macroporosity, MP; soil organic matter, SOM; and soil moisture, SM) measured in Trial I. The first two axes explained 76.4% of the variance.

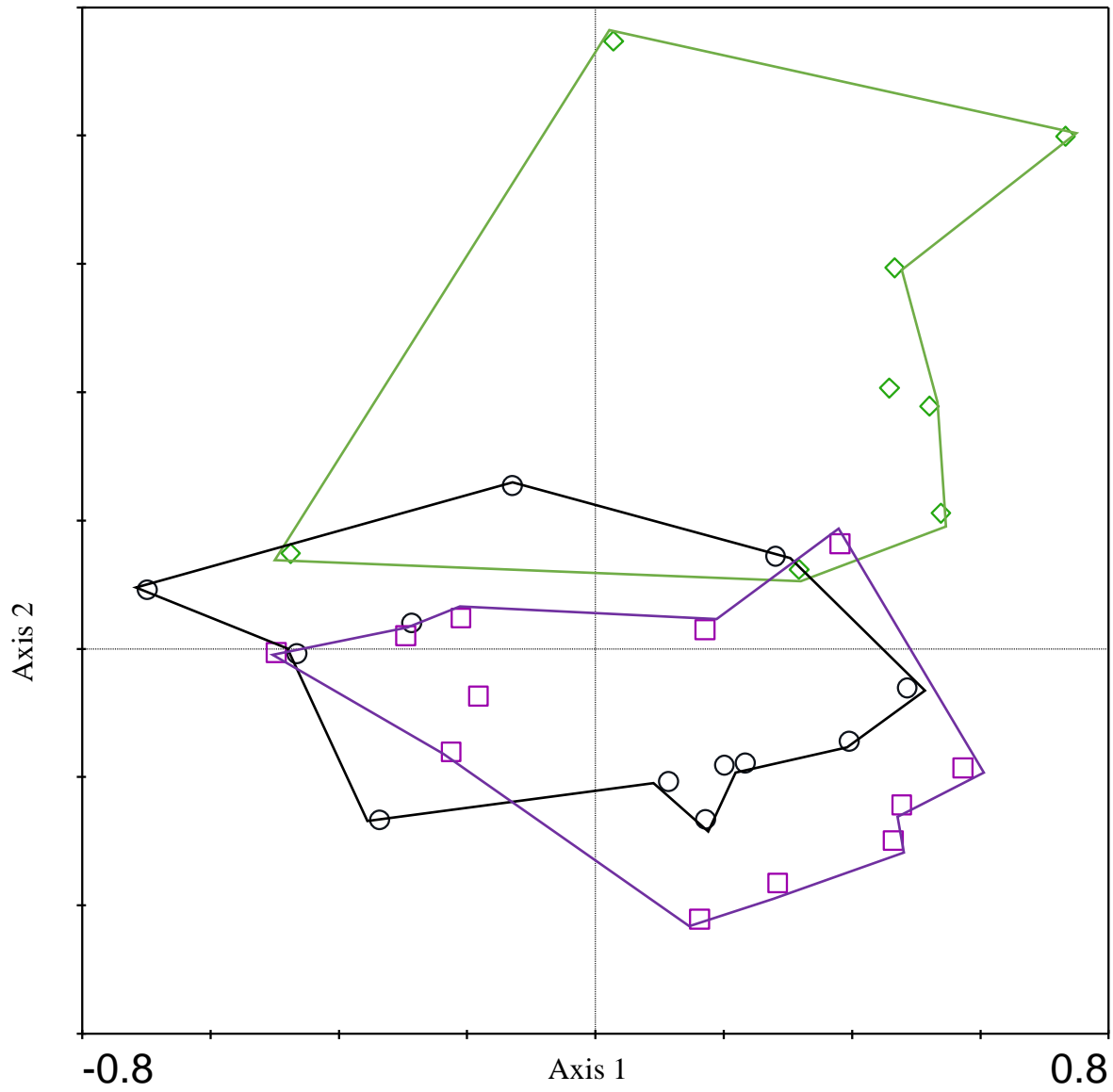


Fig. 2.6. Scattered plots of position of samples and treatment of no-till black oat (BO), bare ground (BG), and solarization (SOL) on the first two canonical axes in Trial I. Boundary line for sample values are color coordinated with treatment color: BG = black, BO = purple, and SOL = green.

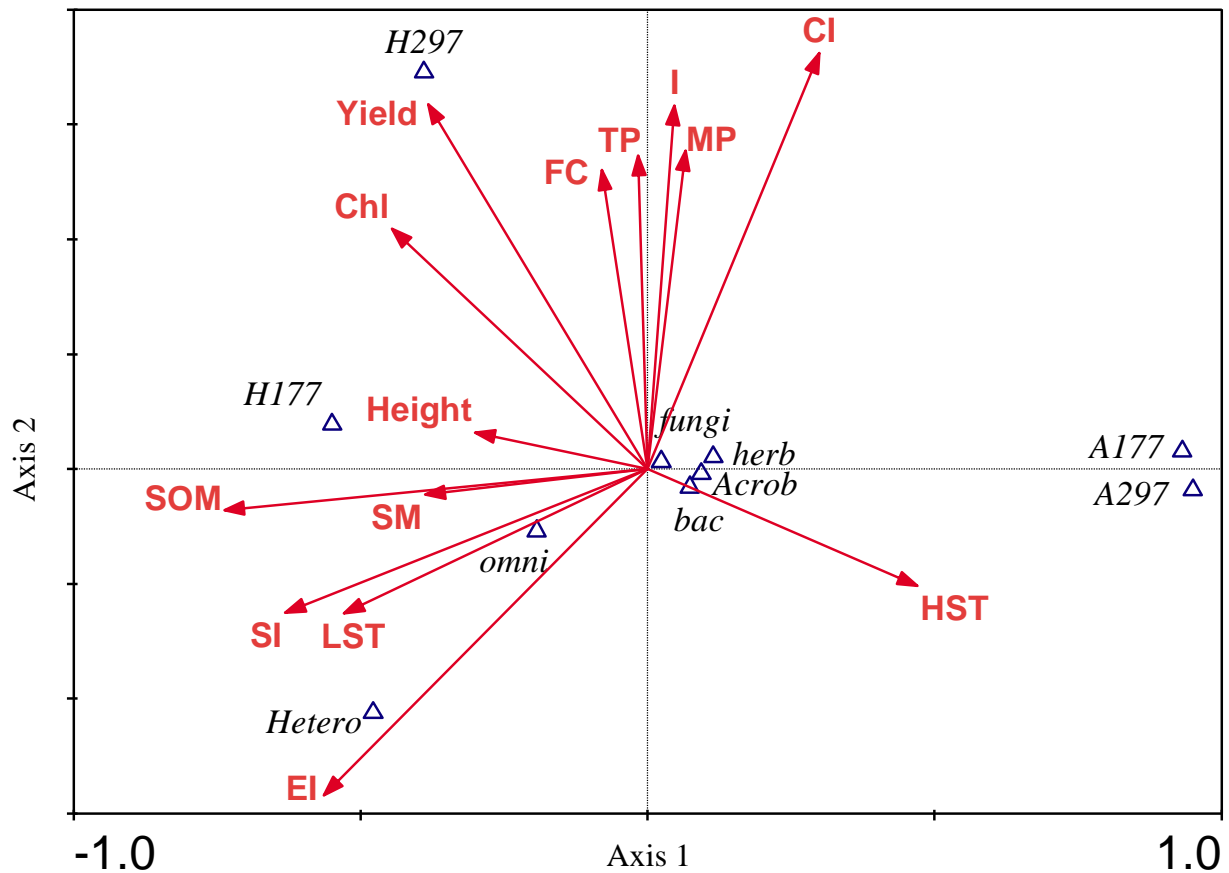


Fig. 2.7. Ordinance diagram depicts the first two canonical axes of a canonical correspondence analysis between species variables (abundance of *Heterorhabditis*, Hetero; abundance of *Acrobeloides*, Acrob; occurrence of *Heterorhabditis* from field cage larva bait assay H297 and H177, occurrence of *Acrobeloides* from field cage larva bait assay A297 and A177; and abundance of nematode trophic groups including bacterivores, bac; fungivores, fungi; herbivores, herb; and omnivores, omni) and environmental variables (channel index, CI; enrichment index, EI; structure index, SI; field capacity, FC; infiltration, I; total porosity, TP; macroporosity, MP; soil organic matter, SOM; soil moisture, SM; lowest soil temperature, LST; highest soil temperature, HST; yield; chlorophyll, Chl; and Height) measured in Trial II. The first two axes explained 76.9% of the variance.

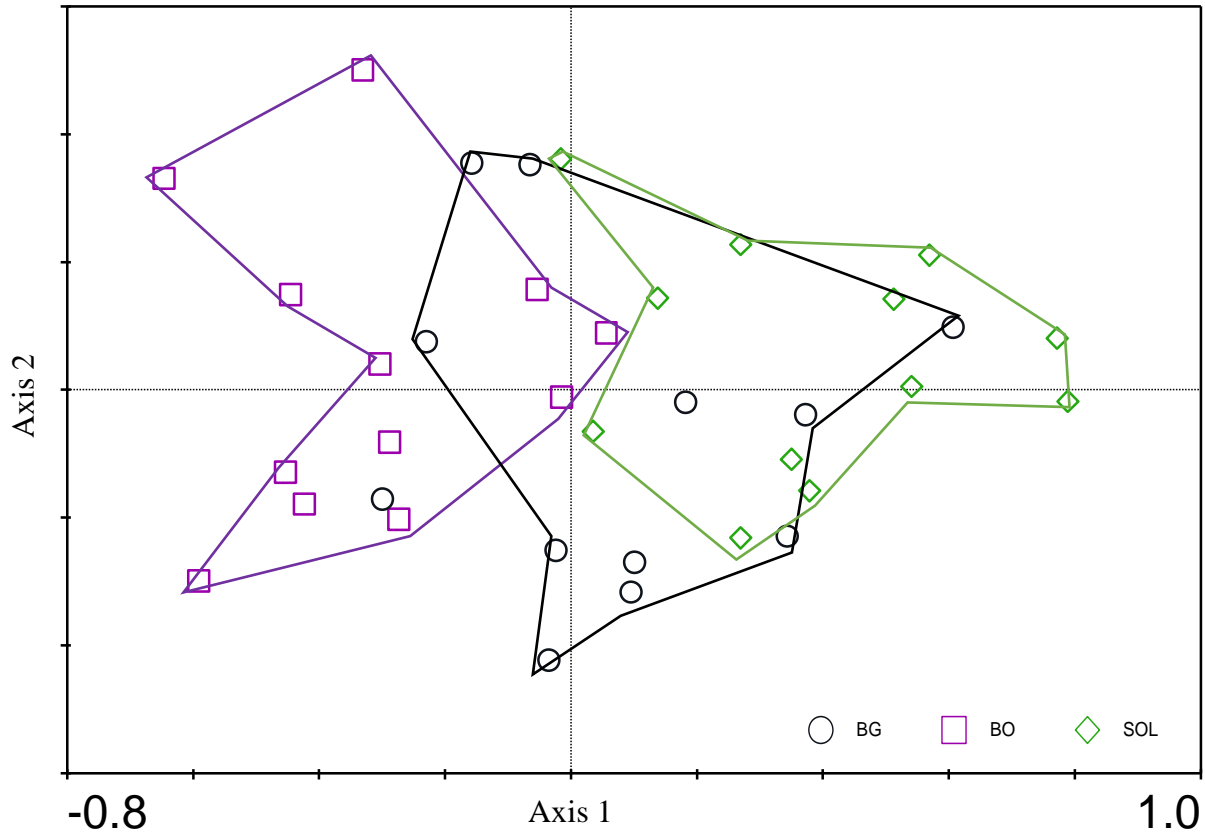


Fig. 2.8. Scattered plots of position of samples and treatments of no-till black oat (BO), bare ground (BG), and solarization (SOL) on the first two canonical axes in trial II. Boundary line for sample values are color coordinated with treatment color: BG = black, BO = purple, and SOL = green

CHAPTER 3

ENHANCEMENT OF INDIGENOUS ENTOMOPATHOGENIC NEMATODES AND ENTOMOPATHOGENIC FUNGI BY OIL RADISH (*RAPHANUS SATIVUS* SSP. *OLEIFERA*) NO-TILL COVER CROPPING IN A CORN (*ZEA MAYS*) AGROECOSYSTEM

Abstract

Two field experiments were conducted in Oxisol to examine if planting oil radish (*Raphanus sativus* ssp. *oleifera* cv. Sodbuster) as cover crop in a no-till system could improve soil conditions for indigenous entomopathogenic nematodes (EPN), *Heterorhabditis* sp., and an entomopathogenic fungus (EPF), *Metarhizium anisopliae*, in a corn (*Zea mays*) agroecosystem. The first experiment examined the effect of oil radish ages on infective juveniles (IJs) of *Heterorhabditis*. Oil radish at 6- and 8-week old had higher abundance of EPN than 2- and 4-week old oil radish and the bare ground control ($P \leq 0.05$), resulting in higher rates of infections in the larvae bait assays ($P \leq 0.05$). In the second experiment, two field trials were conducted to compare pre-plant treatments of 1) OR as a cover crop followed by no-till planting 2) bare ground (BG) followed by conventional tillage, and 3) conventional tillage followed by soil solarization (SOL) on various edaphic factors, EPNs and EPF abundance and infectivity, and soil food web in corn fields. No-till with OR increased volumetric soil moisture, field capacity, and soil organic matter than BG and SOL ($P \leq 0.05$) in both trials, but only increased soil food web enrichment ($P \leq 0.05$) compared to BG and SOL in Trial II. Abundance of *Heterorhabditis* in

OR was greater than that in BG ($P < 0.05$) only at cover crop termination in Trial I, but was consistently more abundant throughout the corn crop in Trial II. However, no-till cover cropping with OR failed to increase EPN infectivity in the field during the corn growing season. The lack of consistent correlations from multivariate correspondence analysis of EPN infectivity to EPN abundance, favorable edaphic factors, and healthy soil food web suggested that oil radish did improve conditions that can enhance EPN abundance, but produced biotoxic compounds that reduced EPN foraging efficiency.

Keywords: conservation biological control, conservation tillage, *Heterorhabditis*, *Metarhizium anisopliae*, herbivore induced plant volatiles (HIPVs), solarization

3.1 Introduction

A concept of in-field augmentation of indigenous entomopathogens including entomopathogenic nematodes (EPN) or entomopathogenic fungi (EPF) through manipulations of field conditions is known as conservation biological control (CBC) (Stuart *et al.*, 2008). This has been recognized as a more practical and cost effective biological control strategy especially because of Hawaii's stringent quarantine regulation to introduce EPN (Myers *et al.*, 2015). Development of CBC usually aims at improving reproduction, persistency, and infectivity of natural enemies in an ecosystem (Fuxa, 1987). Reproduction of EPNs is especially tenuous as they are obligate parasites, requiring the infection of an insect to produce offspring (Kaya and Gaugler, 1993). On the other hand, EPF are hemibiotrophic, having a parasitic phase followed by a saprophytic phase. Therefore, EPF can continue their life cycle as a saprophyte after infection

(Pell *et al.*, 2009). Since both of these insect pathogens have a broad host range (Kaya and Gaugler, 1993; Stuart *et al.*, 2006), an effective CBC approach to use entomopathogens would manipulate the agroecosystem in favor of attracting non-economic damaging insect hosts to serve as alternative hosts for EPN reproduction, including non-phytophagous arthropods. Should the alternative insect hosts reach an abundant level, a trophic cascade effect would impose on entomopathogens to reduce population of target pests and thus increase crop productivity (Denno *et al.*, 2008).

Oil radish (*Raphanus sativus* ssp. *oleifera* cv. Sodbuster) was selected to enhance EPNs and EPF. Oil radish is well known for its benefits of improving soil health by bio-drilling (Chen and Weil, 2010; Williams and Weil, 2004), nitrogen and phosphorous scavenging (Miller *et al.*, 1994), and weed suppression (Lawley *et al.*, 2011; Stivers-Young, 1998). More importantly for this research, small insect larvae associated with brassicaceous crops like cabbage fly (*Delia radicum*), pollen beetle (*Meligethes stephens*), cabbage seed weevil (*Ceutorrhynchus assimilis*), and cabbage moth (*Mamestra brassicae*) can support the reproduction of EPN (Nielsen and Philipsen, 2004). However, this phenomenon has not been examined in field conditions. In a laboratory study, *Heterorhabditis zealandica* were attracted to roots of germinating oil radish but not rutabaga (*Brassicae napus* var. *napobrassica*) seedlings. Increasing the germination rate of seeds increased the attraction of *H. zealandica* (Lei *et al.*, 1992). Higher counts of *Heterorhabditis* IJs were observed as the age of oil radish increased from 2 to 8 weeks old (Philip Waisen, personal communication). Thus one objective of this study was to examine if planting of oil radish cover crop would attract EPNs.

One possible mechanism of EPN attraction could be due to the release of herbivore induced plant volatiles (HIPVs). HIPVs are plant volatile compounds released from an insect damaged plant known as cues to attract the natural enemies of herbivores (McCormick, *et al.*, 2012). Corn (Rasmann *et al.*, 2005) and citrus (Ali *et al.*, 2012; Filgueiras *et al.*, 2016) release HIPV that recruit EPNs and effectively reduce insect pest by ‘top-down’ regulation (Hiltbold *et al.*, 2010). Above ground HIPVs from brassicaceous crops that recruit insect parasitoids and predators have been well established (McCormick, *et al.*, 2012). However, recruitment of EPNs belowground by oil radish needs further investigation. Jagodič *et al.* (2017) reported that sulfur-containing compounds and glucosinolate breakdown of brassica crops, released through damage of herbivores (Nanner *et al.*, 2015; Crespo *et al.*, 2012), can recruit EPNs.

Unfortunately, glucosinolate breakdown products include biotoxic isothiocyanates that have been reported to disrupt foraging efficiency and infectivity of EPNs when mustard seed meal was incorporated into the soil (Henderson *et al.*, 2009; Ramirez *et al.*, 2009). Similarly, *in vitro* studies of brassicaceous plants such as bittercress (*Barbarea vulgaris*) and arugula (*Eruca vesicaria sativa*) produce isothiocyanates that have fungistatic properties against *M. anisopliae* (Inyang *et al.*, 1999a). Conversely, soluble extracts from brassicaceous crops such as rape seed (*Brassica napus* cv. Falcon), Chinese cabbage (*Brassica rapa* var. *Pekinensis* cv. Tip Top), and turnip (*Brassica rapa* subsp. *rapa* cv. Tokyo Cross) increased conidia germination and virulence of *M. anisopliae* against mustard beetle (*Phaedon cochleariae*) (Inyang *et al.*, 1999b). Unlike other brassicaceous cover crops, oil radish yields lower concentrations of isothiocyanates (Hansen and Keinath, 2013).

Specific objectives of this research were to determine if growing oil radish as a cover crop followed by no-till practice prior to corn planting would 1) augment abundance and enhance persistence of entomopathogens during the cover cropping period; 2) improve soil physical properties in favor of EPN and EPF persistence during corn growth; and 3) enhance a soil food web that promote entomopathogens.

3.2 Materials and Methods

Effects of oil radish age in attracting EPNs: A field experiment was conducted at Poamoho Experiment Station, University of Hawaii (21°32'14.79" N, 158°5'20.29" W) on Oahu from April 2016 to September 2016. The soil type was a well-drained silty clay Oxisol (Wahiawa series, very fine, kaolinitic, isohyperthermic, rhodic haplustox) (Ikawa *et al.*, 1985). 'SodBuster' oil radish was staggered in planting at 2-week intervals from April 14 to May 26, 2016 and resulted in 2, 4, 6, and 8 weeks of growth at termination on 9 June 2016. Each planting age was replicated in 4 plots of 1.2 × 5.5 –m² arranged in a randomized complete block design (RCBD). A control without oil radish was labeled as 0 weeks. Oil radish was terminated by tilling the cover crop into the soil. After terminating oil radish, 'Field Trip' pumpkin (*Cucurbita moschata*) was planted. Soil samples of 6 cores were composited per plot at 0, 4, 8, and 12 weeks after pumpkin planting. Nematodes were extracted from the soil by elutriation (Byrd *et al.*, 1976) and centrifugal sugar flotation (Jenkins, 1964), infective juveniles (IJs) were counted under an inverted microscope.

Soil samples at termination of oil radish were also subjected to a laboratory larvae bait assay (Bedding and Akurst, 1975) using mealworm (*Tenebrio molitor*) larvae to quantify EPN and EPF

infectivity. Soil samples of 120 g (dry weight equivalent) were placed into 140-mm diameter × 15 mm deep petri dishes adjusted to 40% (w/w) soil moisture. Ten mealworm larvae were placed in the dishes of soil for 14 days and observed for EPN and EPF infections. Larva baits were incubated in each dish at room temperature (23 °C), and the mealworm cadavers were examined and collected at 3, 7, and 14 days after the initiation. Individual insect cadavers were surface sterilized in 1% sodium hypochlorite for 1 minute before rinsing and transferring to individual White Traps made of 6-mm diameter Petri dish (White, 1927). White Traps were examined for EPN emergence and EPF infection approximately 2 weeks after establishment. Initial EPN isolates were re-inoculated onto mealworms to perform Koch's postulate (Kaya and Stock, 1997) to determine the nematode's status as an EPN.

Effects of no-till cover cropping with oil radish on EPNs and EPF: Two corn field trials were conducted at Poamoho Experiment Station, University of Hawaii. The first field trial (Trial I) was conducted at 21°32' 12.0228" N, 158° 5' 23.3196" W on Oahu August 2016 to January 2017. Three pre-plant soil treatments established prior to corn planting were 1) oil radish cover crop in a no-till system (OR), 2) soil solarization (SOL) and 3) bare ground (BG), each with 4 replicated plots of 3.7 × 6 m², arranged in a RCBD. The OR treatment was established in field plots with 1-year history of no-till cover crop-vegetable crop rotation. Oil radish seeds were sown on 25 August, 2016 at 9 kg seeds/ha, and grown as a cover crop for 2 months. The cover crop was then terminated on 27 October, 2016 by line trimming. Glyphosate was sprayed on all plots to reduce weed pressure. Tilled plots were covered with a 25-µm thick, ultra-violet light stabilized, low density transparent polyethylene mulch (ISO Poly Firms, Inc., Gary Court, SC). Soil solarization was used as a negative control as it was expected to kill majority of the

entomopathogens after reaching a lethal temperature of 37 °C (Stuart *et al.*, 2006). Soil temperature was measured with temperature probes (WatchDog B-series button data logger, Spectrum® Technologies, Aurora, IL) at a depth of 5 and 15 cm in one plot of each treatment during the cover cropping period. Soil in the BG treatment remained fallow with weeds followed by rototilling prior to corn planting. In each plot, 6 rows of ‘Hawaiian SuperSweet # 9’ corn were planted on 35.5-cm row spacing at 23 cm between plants within the row at a rate of 15,240 seeds/ha on 3 November 2016. The crop was fertilized at a rate of 130 kg of nitrogen, 56.7 kg of phosphorus, and 108 kg of potassium per ha. Crops were drip irrigated uniformly throughout the field based on crop demand. Corn was harvested and the experiment terminated at 12 weeks after corn planting.

Trial II: A second field trial (Trial II) was conducted in a different field from Trial I with an 8-year history of no-till cover crop-cash crop rotation at Poamoho Experiment Station, University of Hawaii (21°32’ 9.6756” N, 158° 5’ 21.8796” W). Slight treatment modification from Trial I were established in Trial II. Each treatment plots were 3.7 × 3 m². Four pre-plant treatments installed in Trial II were: 1) oil radish terminated by base cutting with sickles (ORC), 2) oil radish terminated by maceration of plant tissues with a line trimmer (ORM), 3) conventional tillage resulting in bare ground surface (BG), and 4) solarization (SOL) as described in Trial I, except the soil was not tilled prior to covering of solarization mulch. The SOL treatment was conducted in a plot with a previous history of solarization. Since Trial II was conducted during a period not optimal for solarization in Hawaii, soil was not tilled to preserve the soil sterility effect from the last season. Corn was planted on 9 February 2017 and harvested on 4 and 11 May 2017.

Soil analysis: Soil samples were systematically collected from 6 cores from the top 10 cm of soil per plot using a GroundShark Shovel (W.W. Manufacturing, Inc., Bridgeton, NJ) at termination of oil radish right before corn planting, and at 2 and 3 months after corn planting. Soil cores from each plot were composited into a plastic bag and transported to the laboratory for nematode extraction, gravimetric soil moisture measurement, and larva bait assay. The first and last soil samples at planting and harvest were submitted to Agriculture Diagnostic Services Center (ADSC) at the University of Hawaii at Manoa for analysis of total carbon by LECO TruSpec CN (LECO Corporation, Saint Joseph, MI) to estimate soil organic matter (SOM).

Nematode analysis: A 250 cm³ subsample was taken from each soil sample for nematode extraction via elutriation (Byrd *et al.*, 1976) followed by centrifugal floatation method (Jenkins, 1964). Due to concerns with efficacy of extraction of *Heterorabditis*, dauer larvae by centrifugal floatation method, 60 cm³ of soil from each sample was incubated in Baermann funnel (Baermann, 1917) for 3 days to extract IJs. Nematode counts were then extrapolated to 250 cm³ of soil prior to data analysis.

All nematodes from elutriation and centrifugal floatation method were identified to the genus whenever possible with the aid of an inverted microscope (Leica DM IL LED, Wetzlar, Germany) and subjected to nematode community analysis as bioindicators of soil food web conditions. Nematodes were assigned to one of the trophic groups: bacterivores, fungivores, herbivores, omnivores, and predators as described by Yeates *et al.*, (1993). *Filenchus* and *Tylenchus* were designated as fungivore (Okada and Kadota, 2003). *Prismatolaimus* was classified as a bacterivore instead of substrate digester (Yeates *et al.*, 1993). Taxonomic families were assigned to the 1-5 c-p scale of Bongers and Bongers (1998). Richness was calculated as

the total number of taxa per sample. Dominance (λ) was calculated as $\lambda = \sum(p_i)^2$, where p_i is the proportion of each taxon present (Simpson, 1949) and diversity was calculated as $1/\lambda$. Fungivore (F) to bacterivore (B) ratio was calculated as $F/(F + B)$ to characterize the dominant decomposition pathways (Freckman and Ettema, 1993). Nutrient enrichment in the soil was indicated by weighted abundance of bacterivores with c-p value of one (Ba_1) and fungivores of c-p value of two (Fu_2) calculated as enrichment index (EI) using the equation $[e/(e + b)] \times 100$, in which e is total weight abundance of Ba_1 and Fu_2 and b is the weight abundance of nematodes in the basal food web consisting of Ba_2 and Fu_2 . Characterization of fungal or bacterial decomposition pathway was represented by channel index (CI), calculated as $[0.8Fu_2/e] \times 100$ (Ferris *et al.*, 2001). Maturity index (MI) represents nematode fauna weighted by c-p values and calculated as $\sum_{i=1}^n v_i f_i$ in which v_i is the c-p value and f_i is the frequency of the taxon (Bongers, 1990). Resilience, speciousness, and abundance of trophic links associated with the soil food web structure is represented by structure index (SI) calculated as $[s/(s + b)] \times 100$, in which s is the weight abundance of free living nematodes with c-p values higher than 2 (Ferris *et al.*, 2001).

EPN larva bait assays: Laboratory larvae bait assays and field cages larvae bait assays were used to estimate infectivity of EPN. Two types of field cages modified from McCoy *et al.*, (2007) were made from 297- μ m or 177- μ m pore strainers for spraying equipment (Kleen-rite corp., Columbia, PA) sealed with lids from FalconTM tubes (ThermoFisher Scientific Inc., Waltham MA) to cover both the top and bottom openings. DuckTape® (ShurTech brands, Avon, Ohio) was wrapped around the rims of the top and bottom openings to fill the gap between the screen and FalconTM tube lid. Each field cage contained 5 mealworm larvae close to the final instar.

Field cages were filled three quarter-full with field soil and buried to about 15 cm deep near the center of each field plot for 2 days every other week for a total of 7 sampling dates throughout the corn crop. The 177- μ m pore field cages were used to block the intrusion of ants, thus extending the exposure period to 7 days to allow for detection of EPF infections.

Soil water properties: Gravimetric soil moistures for each soil sample collected for larvae bait assay were measured by drying the soil in the oven for 48 hours at 70°C. Soil water potential in the corn rhizosphere was monitored using WatchDog Watermark Soil Moisture Sensor (Spectrum Technologies, Inc., Aurora, IL) every hour throughout the corn growth. In addition to Watermark water potential probe, a WaterScout SM 100 Soil Moisture Sensor (Spectrum Technologies Inc., Aurora, IL) was also installed at the same locations to monitor volumetric soil moistures hourly. In addition, FieldScout TDR 100 Soil Moisture Meter was used to measure volumetric soil moistures weekly with 12-cm rods at the corn rhizosphere from 3 randomly selected spots in each plot. The TDR soil moisture meter was calibrated from taking readings of undisturbed soil cores in which their volumetric soil moisture was determined.

Soil infiltration rate was measured for each plot by single-ring infiltration method (Bouwer, 1986) using a 25.4-cm-diameter metal ring (infiltrator). Water level inside the infiltrator was maintained at 1 cm for 30 minutes for each plot. Steady infiltration rate was derived by the slope of the linear regression line of volume of water infiltrated between 500 to 1800 seconds after initiation of the infiltration test. Cumulative infiltration was also derived from data at 500 to 1800 seconds. FieldScout TDR 100 Soil Moisture Meter was used to measure gravitational soil moisture immediately after the completion of the infiltration test. The infiltration site was then covered with a plastic bag to avoid evaporation and precipitation. Bulk density (Db) was

measured 2 days after infiltration with a 10-cm diameter polyvinyl chloride (PVC) core to a depth of 10 cm and determined by a procedure described by Blake (1965). Soil porosity (Vomocil, 1965) was calculated as $[1 - (BD/2.85)]$ assuming 2.85 g/cm^3 as the standard soil particle density. Field capacity of the soil was measured from the volumetric soil moisture of the Db core (Peters, 1965). Macroporosity was measured from subtracting volumetric soil moisture at field capacity from total porosity. In Trial II, soil water properties from ORM and ORC were combined and refer to as a no-till oil radish treatment (OR).

Soil temperature: Soil temperature probes (WatchDog B-series button data logger, Spectrum® technologies, Aurora, IL) were buried to a 5-cm depth on the day of corn planting in the center of each plot. Soil temperature recording was at hourly intervals.

Thrips assay: Yellow sticky traps ($13 \times 7.5 \text{ cm}^2$) were installed in each plot hanging at 30 cm above ground. Sticky traps were left in the field for 1 week at the 6th, 9th, and 12th week of corn growth. The yellow sticky traps were brought back to the laboratory, observed under a dissecting microscope (Leica© M125, Buffalo Grove, IL) and counted for numbers of thrips per sticky trap.

Weed management monitoring: Weeds in each plot were treated with glyphosate herbicide once prior to corn planting followed by manual removal every 2 weeks after corn planting. Weeding ceased after 4 weeks of critical-weed-free period. Time of weed management per person was recorded for each plot as a measure of weed pressure.

Corn growth and yield: Corn height and chlorophyll content were collected monthly from 3 plants per plot. Corn height was measured from the top of the plant to the soil level. Chlorophyll

was measured with a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) measuring the third leaf from the top. Leafhoppers (*Dalbulus maidis*) were controlled with Sevin® (Novasource, Phoenix, AZ). Corn was harvested on the 12 weeks after planting. Corn was harvested from the middle 4 rows of each plot. Height, chlorophyll, and yield of ORM and ORC plots were combined and referred to as oil radish treatment (OR).

Statistical analysis: Data consisting of one sampling date were subjected to one-way analysis of variance (ANOVA) using PROC GLM in SAS 9.3 (SAS Inc, Cary, NC). Nematode and EPF abundance data were log-transformed before ANOVA. Homogeneity of variance over time was tested for data with multiple sampling dates. If there was no significant interaction between sampling date and treatment effect, data were subjected to repeated measures analysis. Means were separated using Waller-Duncan k -ratio ($k=100$) t -test wherever appropriate. Only true mean values were presented. Canonical correspondence analyses were used to deduce relationships between abundance of IJs with abundance and infectivity of *Acrobeloides*; nematode trophic groups, EI, SI, and CI; soil properties as volumetric soil moisture (SM), volumetric field capacity (FC), soil organic matter (SOM), total soil porosity (TP), macroporosity (MP), water infiltration rate (I), lowest daily soil temperature (LST), and highest daily soil temperature (HST); and corn growth parameters as yield, height, and chlorophyll content (Chl) using CANOCO 4.5 for Windows.

3.3 Results

Effects of oil radish age in attracting EPNs: Since there was no treatment and date interaction based on ANOVA, abundances of *Heterorhabditis* IJs from the 4 sampling dates

after oil radish termination were combined in a repeated measure analysis. IJs were greater in abundance when oil radish was 6- and 8-week old compared to no oil radish (0 week) and 2-week old treatment ($P \leq 0.05$, Fig. 3.1A). Higher infectivity of EPN on mealworms were observed on soil collected from 6- and 8-week old oil radish than 0 week treatment ($P < 0.05$, Fig. 3.1B).

Effects of no-till cover cropping with oil radish on EPNs and EPF:

Pre-plant conditions: Oil radish fresh biomass in Trial I was 21 tons/ha, whereas biomass in Trial II was 79 tons/ha. In Trial I, solarized plots reached a maximum soil temperature of 56.5 °C at 5 cm and 40.5 °C at 15-cm soil depth. The 5-cm depth had a duration of 429 hours above the EPN lethal temperature of 37 °C, while 15-cm depth had a duration of 25 hours above the lethal temperature, respectively during the solarization period. Before corn planting, BG plots had a maximum soil temperature exceeding the lethal temperature of EPN, achieving maximum temperature of 40.5 °C and 38.0 °C at the 5-cm and 15-cm depth, with a total duration of 25 and 2 hours above 37 °C for 5-cm and 15-cm depth, respectively. OR plots did not accumulate any hours above EPN lethal temperature. In Trial II, since pre-plant treatment was initiated in the cooler months with more frequent cloud cover, soil solarized plots reached a maximum soil temperature of only 36.0 °C at 5-cm and 33 °C at 15-cm depth. Temperatures did not reach above the lethal temperature of EPNs at either 5-cm or 15-cm depth. However, SOL plots were successfully solarized in the previous year. BG and OR also did not reach lethal temperatures.

Soil physical properties at post-plant: Soil bulk density was consistently heavier with less total porosity in OR than BG and SOL for both trials ($P \leq 0.05$; Table 3.1). Infiltration rate

was consistently faster in BG and SOL than OR at planting for both trials ($P \leq 0.05$, Table 3.1 and 3.2) but the effect dissipated at corn harvest ($P > 0.05$). In Trial I, macroporosity was higher ($P \leq 0.05$) in BG and SOL than OR only at planting, but not at harvest ($P > 0.05$, Table 3.2). In Trial II, OR had lower macroporosity throughout the corn cropping cycle ($P \leq 0.05$; Table 3.2). Although OR generally maintained a higher field capacity in Trial I ($P \leq 0.05$; Table 3.1), a treatment and date interaction occurred in Trial II and was only higher than BG and SOL during the initial sampling at planting ($P \leq 0.05$; Table 3.2). Although an interaction ($P \leq 0.05$) between sampling time and treatment effect occurred for volumetric soil moistures in both trials, all sampling dates had higher soil moisture in OR than SOL and BG ($P \leq 0.05$). In Trial II, ORM resulted in lower soil moisture content than ORC for only Week 1 and 5 in Trial II. Repeated measure of all soil moisture readings over times revealed that OR had greater volumetric soil moisture than BG and SOL in Trial I ($P \leq 0.05$; Table 3.1), whereas ORC/ORM had greater volumetric soil moisture than BG. SOL had the lowest soil moisture in Trial II ($P \leq 0.05$). OR had higher SOM than BG and SOL only at planting ($P \leq 0.05$; Table 3.2), but not at harvest. In Trial II, OR was consistently higher in SOM than BG and SOL ($P \leq 0.05$; Table 3.1).

Maximum soil temperature reached during the corn growing period was higher in BG and SOL than OR in Trial I ($P \leq 0.05$, Table 3.3) but no difference was detected in Trial II ($P > 0.05$). BG and SOL also had more hours of the higher daily soil temperature ranging from 30-35 °C than OR in Trial I. In Trial II, only BG had more hours of temperature between 30-35 °C than ORC ($P \leq 0.05$; Table 3.3). In addition, ORC maintained more hours of temperatures between 20-25 °C than BG and ORM ($P \leq 0.05$; Table 3.3). The 7-day mean hourly soil temperature during the morning and afternoon were different among treatments for the first 3 weeks in Trial I

and the first 5 weeks in Trial II (Fig. 3.2). Although temperatures during the cooler morning hours were not different among treatments, OR resulted in lower temperatures during the warmer afternoon hours (12 to 7 pm) than BG and SOL in both trials ($P \leq 0.05$). However, during weeks 6 to 11 in Trial I, OR was consistently warmer than BG and SOL regardless of the hour of the day ($P \leq 0.05$). None-the-less, all average temperatures during this corn growing period did not exceed 24.2°C. In Trial II, ORM did not maintain lower temperatures than BG and SOL in the afternoon after Week 3. Furthermore, ORM was consistently warmer than BG and SOL from weeks 6 to 10 ($P \leq 0.05$), but ORC was consistently cooler than ORM, BG, and SOL from weeks 6 to 9 and weeks 11 to 12 ($P \leq 0.05$) regardless of the hour of the day.

EPN and EPF abundance and infectivity: All the EPNs found in both trials were undescribed species of *Heterorhabditis*. In Trial I, abundance of *Heterorhabditis* IJs was greater in OR than BG and SOL at 0 weeks ($P \leq 0.05$). However, abundance of *Heterorhabditis* in OR decreased over time and did not differ from BG at 6 and 12 weeks ($P > 0.05$, Fig. 3.3A). In Trial II, due to absence of interaction between treatment and sampling time, repeated measure over the 3 sampling dates revealed that *Heterorhabditis* abundance was greater in ORC and ORM than BG and SOL ($P \leq 0.05$, Fig. 3.3B) with no IJs detected in SOL throughout the cropping season.

Based on the laboratory larva bait assays repeated measures over 3 dates, no interaction between treatment and sampling time were observed based on ANOVA ($P > 0.05$), thus data were combined (Table 3.4). Overall, pre-plant treatments had no effect on total mealworm mortality in Trial I. SOL had lower mealworm mortality than BG, ORC, and ORM in Trial II ($P \leq 0.05$). However, incidence of mealworms infected by *Heterorhabditis* were greater in OR than SOL in Trial I ($P \leq 0.05$), while BG and OR was greater than SOL in Trial II ($P \leq 0.05$). In

contrast, occurrence of *Acrobeloides* from mealworm cadavers was higher in SOL than BG and OR in Trial I ($P \leq 0.05$) though there was no differences in Trial II ($P \leq 0.05$). Incidence of mealworm infected by *M. anisopliae* was only higher in BG than OR and SOL in Trial I ($P \leq 0.05$).

Based on the 297- μm pore field cage larvae bait assay, higher incidence of mealworm infected by *Heterorhabditis* were detected in OR and BG than SOL in Trial I ($P \leq 0.05$; Table 3.5) and more infection were detected in ORC than SOL ($P \leq 0.05$) but were not different between BG and oil radish treatments in both trials. Based on the 177- μm pore field cage assay, no difference in incidence of mealworms being infected with *Heterorhabditis* was observed among treatments ($P > 0.05$) in both trials. In contrast, the incidence of *Acrobeloides* recovery from field cage assays was higher in SOL than BG, ORC, and ORM from 297- μm pore field cage ($P \leq 0.05$) and higher than ORC in the 177- μm pore field cage ($P \leq 0.05$) in Trial II.

Effects on soil food web: Although an interaction between sampling dates and treatment effects was significant for abundance of bacterivore and fungivore, richness, and CI, only means from the treatment effect over the 3 sampling dates were presented in Table 3.6. Overall, the impact of OR in no-till system on the nematode community was not different from BG in Trial I ($P > 0.05$; Table 6), except for lower abundance of bacterivores in OR than BG at harvest (12 wks) and higher abundance of fungivores at corn planting (0 wks) in OR than BG ($P \leq 0.05$, Table 3.7). Richness was greater in OR than BG only at planting ($P \leq 0.05$, Table 3.7). In contrast, ORC and ORM in Trial II had lower abundance of *Meloidogyne*, *Pratylenchus*, and fungivores, as well as lower % herbivores than BG throughout the experiment ($P \leq 0.05$; Table 3.6). Moreover, both ORC and ORM increased EI, reduced F/(F+B), CI and richness compared

to the BG control with no effects on SI within the corn cropping season in Trial II (Table 3.6). Only at planting did abundance of bacterivores from ORM exceed that of BG and ORC ($P \leq 0.05$, Table 3.7). The effect of different termination techniques showed lower abundance of fungivores and bacterivores in ORC than ORM ($P \leq 0.05$; Table 3.6).

Although SOL only reduced abundance of bacterivores and richness at planting compared to BG and OR ($P \leq 0.05$; Table 3.7), throughout the corn growing season in Trial I, SOL reduced EI while increased CI compared to BG and OR ($P \leq 0.05$; Table 3.6). In Trial II, SOL reduced abundance of bacterivores compared to BG and ORM. SOL also had the lowest numbers of fungivores and % fungivores among treatments throughout the crop growing season ($P \leq 0.05$; Table 3.6). Conducting SOL during cooler season in Trial II did not suppress % herbivores nor affect the SI compared to that in the BG, but it did reduce EI ($P \leq 0.05$) throughout the trial.

Pre-plant soil treatments also affected nematophagous fungi but only in Trial II where *Catenaria* was observed in the last sampling date (Table 3.6). *Catenaria* had a higher occurrence in BG and ORC than SOL ($P \leq 0.05$). Total nematophagous infections including *Lagenidium* and *Rhizophidium* were greater in ORC than SOL ($P \leq 0.05$).

Relationship between EPN, edaphic factors and soil health conditions: Canonical Correspondence Analyses (CCA) between species variables that include EPN abundance and activity, abundance of other free-living nematodes in the soil with environmental variables that include nematode community indices and soil edaphic factors from Trial I and Trial II were depicted in an ordination diagrams in Fig. 4 and Fig. 6, respectively. In Trial I, the first two canonical axes explained 81.8% of the variance, while in Trial II, they explained 95.0% of the variance. In Trial I, *Heterorhabditis* abundance

(*Hetero*) were positively correlated with soil moisture (SM) and enrichment index (EI), but negatively correlated with channel index (CI), total porosity (TP), and macroporosity (MP). Strangely, *Heterorhabditis* infectivity from 177- μ m pore field cage (H177) was positioned opposite of *Heterorhabditis* abundance. The 297- μ m pore field cage were not included in this analysis due to low detection rate of EPN infected mealworms (7.2 %). *Heterorhabditis* abundance had no clear relationship with soil temperatures and corn growth parameters were all correlated with SI but not with EI and CI in both trials (Fig. 3.4 and Fig. 3.6). In Trial II, *Heterorhabditis* abundance and its infectivity in 297- μ m pore field cage was positively correlated with parameters associated with soil health and plant growth parameters including soil organic matter (SOM), field capacity (FC), total porosity (TP), macroporosity (MP), structure index (SI), corn height (Height), corn yield (Yield), and chlorophyll content (Chl), while negatively correlating with the highest daily soil temperatures (HST). However, *Heterorhabditis* infectivity from 177- μ m pore sieve field cage were positioned on the opposite side of the second axis. The 177- μ m pore field cage positively correlated with EI and lowest daily soil temperatures (LST) and negatively correlated with steady infiltration (I) and CI. *Acrobelloides* from 297- μ m pore field cages were negatively correlated with SM and *Heterorhabditis* infectivity from 177- μ m pore sieve field cage. *Acrobelloides* from 177- μ m pore field cages was positively correlated with CI and soil infiltration (I) and negatively correlated with EI and LST. When sampling points were plotted against the canonical axes, SOL was segregated from OR along the second axis, while BG are plotted in-between SOL and OR in Trial I (Fig. 3.5). In Trial II, there was no apparent separation of sampling points among treatments except for ORC and BG along the second axis, though three sampling points were overlapped between these treatments (Fig. 3.7).

Corn growth, yield and weed pressure: Yields were not different among treatments for both trials ($P > 0.05$; Table 3.8). Corn heights were not different in Trial I, but SOL had higher corn heights than BG and OR in Trial II ($P \leq 0.05$). Weed pressure, represented by time of weeding, was less in SOL than BG and OR in both trials ($P \leq 0.05$). In Trial I, chlorophyll content was greater in BG than OR only at Week 6 ($P \leq 0.05$; Table 3.9). In Trial II, BG had the lowest chlorophyll content with OR higher and SOL at the highest at Wee 6 ($P > 0.05$). For Week 9 and 12, OR was consistently higher in chlorophyll content than BG ($P \leq 0.05$) but was not different from SOL.

3.4 Discussion

Effect of no-till OR cover cropping on entomopathogens: Planting OR in a no-till field increased abundance of *Heterorhabditis* sp. in the soil. In the oil radish age experiment, occurrence of *Heterorhabditis* from larva bait assay suggested that planting oil radish as cover crops had potential to augment population densities of EPNs in the field. The longer the oil radish was grown, the higher the abundance of *Heterorhabditis* was detected in the soil, an indication of EPN recruitment by OR. However, based on the two subsequent corn pre-plant treatment trials, augmentation of *Heterorhabditis* by growing oil radish was only transient. Over time the high population of IJs in OR plots dwindled rapidly. Lack of reproduction of this EPN during the growing period could have been 1) lack of insect hosts on corn crop, or 2) inability for EPN to infect insect host. Though total populations of insect host in the soil were not measured, thrips counts from sticky traps were extremely low with an average of only 1-2 thrips/97 cm²

trap and could have limited the reproduction of entomopathogens. In addition, low infectivity rate of *Heterorhabditis* from field cage assays suggested that no-till cover cropping with oil radish does not improve conditions for *Heterorhabditis* compared to conventional tillage despite higher abundance of this EPN in OR.

Oil radish is known to produce low levels of isothiocyanate (Hansen and Keinath, 2013) especially when not incorporated into the soil. It is possible that the indigenous *Heterorhabditis* sp. may be exceptionally sensitive to isothiocyanate and/or other bioactive compounds commonly found in brassicaceous crops such as nitriles, epinitriles, and ionic thiocyanates (Matthiessen and Kirkegaard, 2006). However, the recovery of mobile IJs from soil sample in Baermann funnel after oil radish termination suggested that isothiocyanate from oil radish in no-till may not be nematocidal nor nematostatic. Instead, isothiocyanates might be reducing foraging efficiency of EPN which had been reported by Henderson *et al.* (2009). Disruption of chemotaxis of EPN toward an insect host could result from compounds released by oil radish. Reducing the release of isothiocyanates from oil radish by only cutting the base of the plant (ORC) without macerating the plant cells during the termination of the cover crop (ORM) did not overcome the lack of infectivity of *Heterorhabditis* in oil radish plots despite higher abundance of EPNs in both OR treatments. The attempt to compare rupturing of oil radish cells by line trimming or cell maceration (ORM) to no rupturing by cutting (ORC) did not support the hypothesis that rupturing of plant tissues could increase more HIPV to attract *Heterorhabditis*. There was no difference in abundance or infectivity of *Heterorhabditis* between ORC and ORM in Trial II. However, abundance of *Heterorhabditis* IJs was numerically higher in ORM than ORC, possibly due to more conversion of glucosinolate into isothiocyanates in ORM.

In general, *Heterorhabditis* abundance declined steadily over time after the termination of oil radish. Nematophagous fungal infections were observed from soil extracted nematodes and White Traps and could be a factor in declined population densities of EPNs at the end of the corn growing period. EPNs are highly susceptible to nematophagous fungi as dense EPN populations originating from an insect cadaver can create optimal conditions for epizooticity of nematophagous fungi (Pathak *et al.*, 2017). This phenomena is known as a bottom-up regulation of EPNs by nematophagous fungi (Jaffee and Strong, 2005). Interestingly, all the nematophagous fungi reported in this study are flagellated zoosporic fungi including *Catenaria*, *Lagenidium* and *Rhizophidium* that required higher SM for maneuver. Though their impact might not be significant, the presence of these nematophagous fungi does interfere with the effects of soil treatment on EPNs.

Results from the current study supported the findings of those who reported negative effects of brassicaceous crops on EPF (Inyang *et al.*, 1999a). Laboratory bait assays and field cages in Trial I suggested that OR may be hindering *M. anisopliae* infections. Isothiocyanate released by OR after termination may be responsible for the lack of EPF activity due to its antifungal properties (Matthiessen and Kirkegaard, 2006), though spores or propagules disperser by tillage in BG could have increased the occurrence of *M. anisopliae* in the larvae bait assay in Trial I.

Effects of soil physical properties on entomopathogens: Despite the fact that all soil physical properties measured in no-till OR were more favorable for entomopathogens, field infectivity of EPN and EPF were not enhanced by no-till planting of OR compared to BG. Soil physical properties from OR in Trial I or ORC and ORM in Trial II maintained higher volumetric soil moistures, soil organic matter content, and field capacity than BG and SOL throughout the corn

crop. Soil moisture is known to enhance migration, virulence, and infectivity of EPN (Grant and Villani, 2003; Jabbour and Barbercheck, 2008; Klein, 1990; Shapiro-Llan *et al.*, 2002).

However, higher soil moisture and organic matter in OR did not consistently lead to higher EPNs infectivity in this study among both trials and field cage pore size. Effects of soil treatment on soil temperatures at corn growing season are also not a plausible explanation for lack of effects of no-till OR on EPNs infectivity and abundance. All soil temperatures during growing season never reached the lethal threshold of 37°C for EPNs, except for 1.75 hours in ORM in Trial II. Although OR were able to maintain lower soil temperatures during the hotter afternoon hours than BG and SOL, the effect was transient, only lasting a month after corn planting. Soil surface of OR in both trials appeared bare one month after oil radish termination. This might have exposed the surface of the soil to desiccation and ultra-violet light, which are known to harm EPNs (Shapiro-Ilan *et al.*, 2012). However, ORC consistently maintained lower soil temperatures throughout the corn growing period, yet EPN infectivity was not enhanced. Therefore, the lack of EPN infectivity and the reduction of EPN abundance over time cannot be explained by soil physical conditions as all no-till oil radish treatments created favorable conditions for entomopathogens (*i.e.* higher soil moisture, organic mulch, and lower soil temperatures). Alternatively, biologically active compounds derived from OR may be negatively affecting these entomopathogens in OR plots. Reduction in *Meloidogyne* and *Pratylenchus* populations from oil radish treatments in Trial II was an indication of biofumigant such as isothiocyanates being released by OR hampering the performance of entomopathogens. However, the present of nematophagous fungi may also play a role in interfering the OR effect.

Relationship between EPNs and soil health, soil properties and plant growth: The relationship between EPNs and various plant and soil health parameters were not clear from this study except for a consistent positive correlation between *Heterorhabditis* with EI and negative correlation with CI. EPNs have been reported to correlate with nutrient enrichment (Hoy *et al.*, 2008). Hoy *et al.* (2008) suggested that EPNs and other r-strategist nematode colonizers share similar preferences for soil nutrient enrichment in the agroecosystem. Another explanation is the result of insect cadavers colonized by EPNs become a major source of food for free-living nematodes associated with enrichment (Campos-Herrera *et al.*, 2012) or that an agroecosystem with an enriched soil food web is associated with an attractive habitat of EPN insect host (*i.e.* plant residue).

A consistent correlation between EPNs and other soil properties associated with soil health and plant growth (SM, FC, SOM, TP, MP, Height, Yield, and SI) was lacking among the two trials. Moreover, *Heterorhabditis* abundance and infectivity were not positively correlated, suggesting that higher abundance of *Heterorhabditis* from oil radish did not result in high infectivity of *Heterorhabditis*. Though OR did increase the abundance of indigenous *Heterorhabditis* sp. initially in these field trials before planting a cash crop, OR did not improve EPN infectivity and persistency when coupled with no-till practices in a corn agroecosystem. Despite the fact that OR has lower isothiocyanates production than other Brassicaceous crops (Hansen and Keinath, 2013), results from current field cage assays revealed that OR cover crop resulted in negative impact on EPN infectivity. High counts of EPN in soil at the end of the OR cover crops, but similar infectivity of *Heterorhabditis* on mealworms to BG suggested that some suppressive compounds might have been released from OR after termination.

Whereas mowing or macerating oil radish on soil surface would release HIPVs to attract EPNs (Matthiessen and Kirkegaard, 2007); and whereas the surface organic mulch provided by oil radish in no-till system could also improve conditions for entomopathogens by creating an agroecosystem with diverse alternative insect hosts (Burst, 1991; Hendrix *et al.*, 1986; Kaya and Gaugler, 1993), results from this study did not support the hypothesis that no-till would minimize the negative effect of isothiocyanates from OR against entomopathogens in the soil. Results from the current study also did not totally support the hypothesis that maceration of oil radish tissue would attract more EPNs through the release of HIPV from OR. However, there is a slight indication of reduction in *Heterorhabditis* infectivity from the 297- μm and 177- μm pore field cage assay when OR was macerated (ORM) compared to cut (ORC) probably from the release of more isothiocyanates in ORM. Toxicity from isothiocyanates of mustard cover crop on EPNs has been proven to be evaded by winter kill (Jaffuel *et al.*, 2017). Therefore future research should explore alternative termination techniques like winter kill or herbicide kill to reduce the negative effects of OR on EPN infectivity.

3.5 Conclusion

Although all the soil properties measured in this experiment supported the hypothesis that growing OR as a cover crop in no-till system improved soil conditions favorable for EPNs and EPF (*i.e.* higher soil moisture, lower soil temperature, higher field capacity, and *etc.*), both laboratory and field cage larva bait assays showed low EPN infectivity rates in OR. No-till cover cropping with oil radish only enhanced EPNs abundance transiently. Once OR was terminated,

enhancement of EPNs dissipated despite favorable edaphic factors in the field. Though the effect of pre-plant treatments on EPF was not clear in these trials due to low occurrence, laboratory larva bait assays suggested a negative impact of OR plant residue on EPF in Trial I. Further research is needed to determine the mechanism behind reduced infectivity of EPNs by cover cropping with OR. Potentially, modification of cultural practice that can increase abundance of EPNs without the harmful effects of the oil radish cover crop on EPN and EPF could be integrated with other cover crops such as black oat that can be used to circumvent the allelopathic effect of OR. In addition, other brassicaceous crops may also augment EPNs in the field without the negative impact on foraging efficiency.

3.5 Literature cited

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Table 3.1. Effects of tillage practices on soil properties in corn agroecosystem from no-till oil radish (OR), bare ground (BG), and solarization (SOL) on soil properties.

Soil properties	Trial I			Trial II		
	BG	OR	SOL	BG	OR	SOL
Db ^y (g/cm ³)	1.02 ^z B	1.18 A	1.06 B	1.05 B	1.15 A	1.05 B
FC (θ _v)	32.74 B	35.63 A	33.53 B	34.51 B	36.77 A	33.13 C
MP (%)	31.3 A	23.0 B	29.4 A	28.69 A	22.80 B	30.18 A
SM (θ _v)	29.31 B	36.15 A	28.71 B	32.00 B	35.74 A	31.16 C
SOM (%)	1.20 B	1.61 A	1.15 B	1.11 B	1.37 A	0.96 C
TP (%)	64.06 A	58.59 B	62.95 A	63.20 A	59.57 B	63.31 A

^z Means are repeated measures of data at planting and harvesting with an average of 4 replications (n = 8) for all soil properties except for SM which was measured weekly (n = 52). Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

^y Db = bulk density, FC = Volumetric field capacity, MP = macroporosity, SM= Volumetric soil moisture throughout the corn growing period, SOM = Soil organic matter, and TP = Total porosity.

Table 3.2. Effects of pre-plant treatments of no-till cover cropping with oil radish (OR) compared to conventional tillage (BG) and solarization (SOL) on soil properties at initial corn planting and corn harvest.

Soil properties	Weeks ^z	Trial I			Trial II		
		BG	OR	SOL	BG	OR	SOL
FC ^y (θ _v)	0	-	-	-	33.0 B	37.0 A	32.2 B
	12	-	-	-	36.0 A	36.6 A	34.0 A
I (cm/hr)	0	73.3 ^x A	6.0 B	101.1 A	66.2 A	8.8 B	85.1 A
	12	10.9 A	4.8 A	9.4 A	28.3 A	34.7 A	34.7 A
MP (%)	0	37.4 A	22.7 B	33.1 A	31.0 A	22.1 B	31.5 A
	12	25.8 A	20.7 A	26.1 A	26.4 AB	23.5 B	28.8 A
SOM (%)	0	1.2 B	1.6 A	1.2 B	-	-	-
	12	1.2 A	1.1 A	1.2 A	-	-	-

^z Weeks of corn growth: 0 = planting and 12 = harvest.

^y FC = field capacity, I = infiltration, MP = macroporosity, and SOM = soil organic matter.

^x Means are average of 4 replications. Means in a column followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

Table 3.3. Total hours of soil temperatures in no-till oil radish macerated (OR/ORM), no-till oil radish cut (ORC), bare ground (BG), and solarization (SOL) during corn growing period.

Temp. (°C)	Trial I			Trial II			
	BG	OR	SOL	BG	ORC	ORM	SOL
20-25	1236.8 ^z A	1286.0 A	1144.3 A	1421.5 B	1649.3 A	1481.8 B	1535.5 AB
25-30	148.0 A	185.8 A	181.5 A	454.5 A	300.5 B	438.8 A	329.5 B
30-35	29.0 A	0.8 B	42.0 A	49.5 A	5.75 B	28.0 AB	22.0 AB
35-37	0.0 A	0.0 A	0.3 A	1.3 A	0.0 A	4.8 A	0.3 A
>=37	0.0 A	0.0 A	0.0 A	0.0 A	0.0 A	1.8 A	0.0 A
Max ^y	33.3 A	29.8 B	34.8 A	34.3 A	30.6 A	32.8 A	33.8 A
Min	16.4 A	16.9 A	16.1 A	16.5 A	17.4 A	17.0 A	16.5 A

^z Means are average of 4 replications. Means in a column followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* =100) *t*-test.

^y Max = mean maximum temperature reached throughout the corn growing period, Min = mean minimum temperature reached thought the corn growing period.

Table 3.4. Effects of no-till oil radish macerated (OR/ORM), no-till oil radish cut (ORC), bare ground (BG), and solarization (SOL) on percent occurrence of *Tenebrio molitor* larvae infection by nematodes and/or fungi from laboratory larva bait assays.

Organisms recovered	Trial I			Trial II			
	BG	OR	SOL	BG	ORC	ORM	SOL
	% Mortality						
<i>Heterorhabditis</i>	22.5 ^z AB	33.3 A	11.7 B	35.0 A	36.7 A	36.7 A	5.8 B
<i>Acrobeloides</i>	5.0 B	2.5 B	15.8 A	14.2 A	6.7 A	5.8 A	19.2 A
<i>Metarhizium</i>	22.5 A	4.2 B	3.3 B	25.0 A	28.3 A	43.3 A	27.5 A

^z Means are average of 4 replications. Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* =100) *t*-test.

Table 3.5. Effect of no-till oil radish macerated (ORM), no-till oil radish cut (ORC), bare ground (BG), and solarization (SOL) on percent occurrence of *Tenebrio molitor* larvae in 297- μ m or 177- μ m mesh field cages buried in soil infected by different nematodes and fungi.

Seive pore size (μ m)	Organisms recovered	Trial I			Trial II			
		BG	OR	SOL	BG	ORC	ORM	SOL
297	<i>Heterorhabditis</i>	32.1 A	29.1 A	3.6 B	33.7 AB	36.8 A	31.7 AB	20.4 B
	<i>Acrobeloides</i>	1.4 A	0.8 A	5.8 A	1.5 B	2.3 B	1.5 B	23.8 A
177	<i>Heterorhabditis</i>	6.4 A	9.3 A	5.0 A	16.0 A	29.2 A	20.4 A	17.9 A
	<i>Acrobeloides</i>	0.71 A	0.0 A	1.4 A	5.6 AB	0.0 B	3.3 AB	7.3 A
	<i>Metarhizum</i>	2.9 A	0.0 B	0.7 B	1.5 A	3.1 A	3.1 A	0.8 A

^z Means are average of 4 replications repeated measures over 7 times (n = 84). Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test

Table 3.6. Effect of no-till cover cropping with no-till oil radish macerated (OR/ORM) and cut (ORC) on nematode communities compared to bare ground (BG) and soil solarization (SOL) during corn growing season.

Nematode Community	Trial I			Trial II			
	BG	OR	SOL	BG	ORC	ORM	SOL
<u>Nematode abundance</u>	Nematodes / 250cm ³ soil						
<i>Helicotylenchus</i>	3 ^z A	3 A	3 A	3 A	10 A	16 A	0 A
<i>Meloidogyne</i>	3 A	14 A	0 A	140 A	187 B	74 B	71 B
<i>Paratrichodorus</i>	0 A	0 A	0 A	24 A	5 A	7 A	11 A
<i>Pratylenchus</i>	0 A	0 A	0 A	227 A	20 B	13 B	28 B
<i>Rotylenchulus</i>	356 A	238 A	111 A	633 A	398 A	486 A	604 A
Bacterivore	306 A	458 A	184 B	659 A	419 B	641 A	267 B
Fungivore	227 A	268 A	140 A	410 A	129 C	216 B	75 D
Herbivore	343 A	264 A	118 A	1028 A	619 A	596 A	733 A
Omnivore	28 A	38 A	28 A	47 A	31 A	32 A	32 A
Predator	0 A	1 A	0 A	1 A	3 A	0 A	0 A
<u>Infection by nematophagous fungi</u>							
Total	0 A	0 A	0 A	13 AB	46 A	7 AB	3 B
<i>Catenaria</i> ^y	0 A	0 A	0 A	28 A	80 A	8 AB	0 B
<i>Lagenidium</i>	0 A	0 A	0 A	3 A	3 A	0 A	3 A
<i>Rhizophidium</i>	0 A	0 A	0 A	1 A	11 A	3 A	0 A
<u>Nematode indices</u>							
% Bacterivore	32 A	34 A	32 A	31.3 A	35.8 A	42.3 A	29.4 A
% Fungivore	20 A	21 A	23 A	19.6 A	11.5 BC	14.8 AB	7.8 C
% Herbivore	25 A	26 A	21 A	44.5 B	48.8 AB	39.3 B	59.3 A
% Omnivore	2 A	4 A	4 A	2.3 A	2.8 A	2.3 A	3.2 A
% Predator	0 A	0 A	0 A	0.1 A	0.3 A	0 A	0 A
F/B ^x	48 A	54 A	52 A	36 A	26 B	27 B	23 B
Diversity	7.55 A	6.75 A	6.75 A	6.70 A	10.26 A	6.36 A	5.17 A
Dominance	0.16 A	0.16 A	0.16 A	19 B	19 B	19 B	33 A
Richness	14 A	14 A	10 A	18 A	14 B	14 B	9 C
MI	2.29 A	2.65 A	2.26 A	2.0 AB	1.9 B	1.8 B	2.1 A
EI	56 A	60 A	32 B	57.5 B	71.0 A	72.3 A	13.0 C
SI	20 AB	27 A	19 B	29.1 A	33.0 A	32.8 A	20.3 A
CI	34 B	28 B	75 A	36.4 A	18.7 B	25.4 B	61.1 A

^z Means are repeated measures over 3 dates of 4 replications ($n = 12$). Means in a row followed by the same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t -test based on log-transformation, $\log(x+1)$, for nematode abundance, or square root transformation, $\sqrt{(x + 0.1)}$, for other parameters wherever necessary prior to ANOVA.

^y Occurrence of *Catenaria* was only observed at the last sampling date ($n = 4$)

^x F/B = ratio of the abundance of fungivores to bacterivores (F/F+B), MI = maturity index, EI = Enrichment index, SI = Structure index, and CI = Chanel index.

Table 3.7. Effects of pre-plant soil treatments of no-till oilseed radish macerated (ORM), no-till oilseed radish cut (ORC), bare ground (BG), and solarization (SOL) plots in a corn agroecosystem on nematode parameters by sampling date.

	Week ^y	Trial I			Trial II			
		BG	ORM	SOL	BG	ORC	ORM	SOL
250 cm ³ soil								
Bacterivores	0	485 ^z A	913 A	183 B	745 B	628 B	980 A	322 C
	9	398 A	460 A	363 A	860 A	498 AB	745 A	190 B
	12	605 A	238 B	363 AB	373 A	133 B	198 AB	288 AB
Fungivore	0	143 B	550 A	105 B	-	-	-	-
	9	205 A	148 A	148 A	-	-	-	-
	12	333 A	105 A	168 A	-	-	-	-
Richness	0	13 B	17 A	6 C	-	-	-	-
	9	15 A	14 A	14 A	-	-	-	-
	12	16 A	11 AB	11 B	-	-	-	-
CI	0	15.5 B	26.2 B	100.0 A	-	-	-	-
	9	28.0 A	22.2 A	62.9 A	-	-	-	-
	12	58.3 A	35.3 A	63.5 A	-	-	-	-

^z Means are average of 4 replications. Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test. CI = channel index.

^y Weeks of corn growth: 0 = planting, 6 = mid-growing period, and 12 = harvest.

Table 3.8. Effect of no-till cover cropping with oil radish (OR), conventionally tilled bare ground (BG), and solarization (SOL) on corn plant growth and yield.

Growth parameters	Trial I			Trial II		
	BG	OR	SOL	BG	OR	SOL
Height ^y (cm)	166.9 A	167.6 A	172.9 A	146 B	145 B	162 A
Weeding (Sec/Person)	70.1 A	66.0 A	30.5 B	85 A	88 A	38 B
Yield (kg/ha)	6017 ^z A	5755A	7369 A	6331 A	6815 A	6001 A

^z Means are average of 4 replications. Means in a row followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

^y Height and chlorophyll consist of 3 repeated measures. Weeding consisting of 2 repeated measures.

Table 3.9. Effect of no-till cover cropping with oil radish (OR), conventionally tilled bare ground (BG), and solarization (SOL) on corn chlorophyll content (SPAD unit).

Week	Trial I			Trial II		
	BG	OR	SOL	BG	OR	SOL
6	50.3 ^z A	36.6 B	47.0 AB	28.7 C	34.7 B	39.5 A
9	36.0 A	36.2 A	40.8 A	33.5 B	41.4 A	38.1 AB
12	54.8 A	56.7 A	56.2 A	47.0 B	51.4 A	49.4 AB

^z Means are average of 4 replications. Means in a column followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio ($k = 100$) t-test.

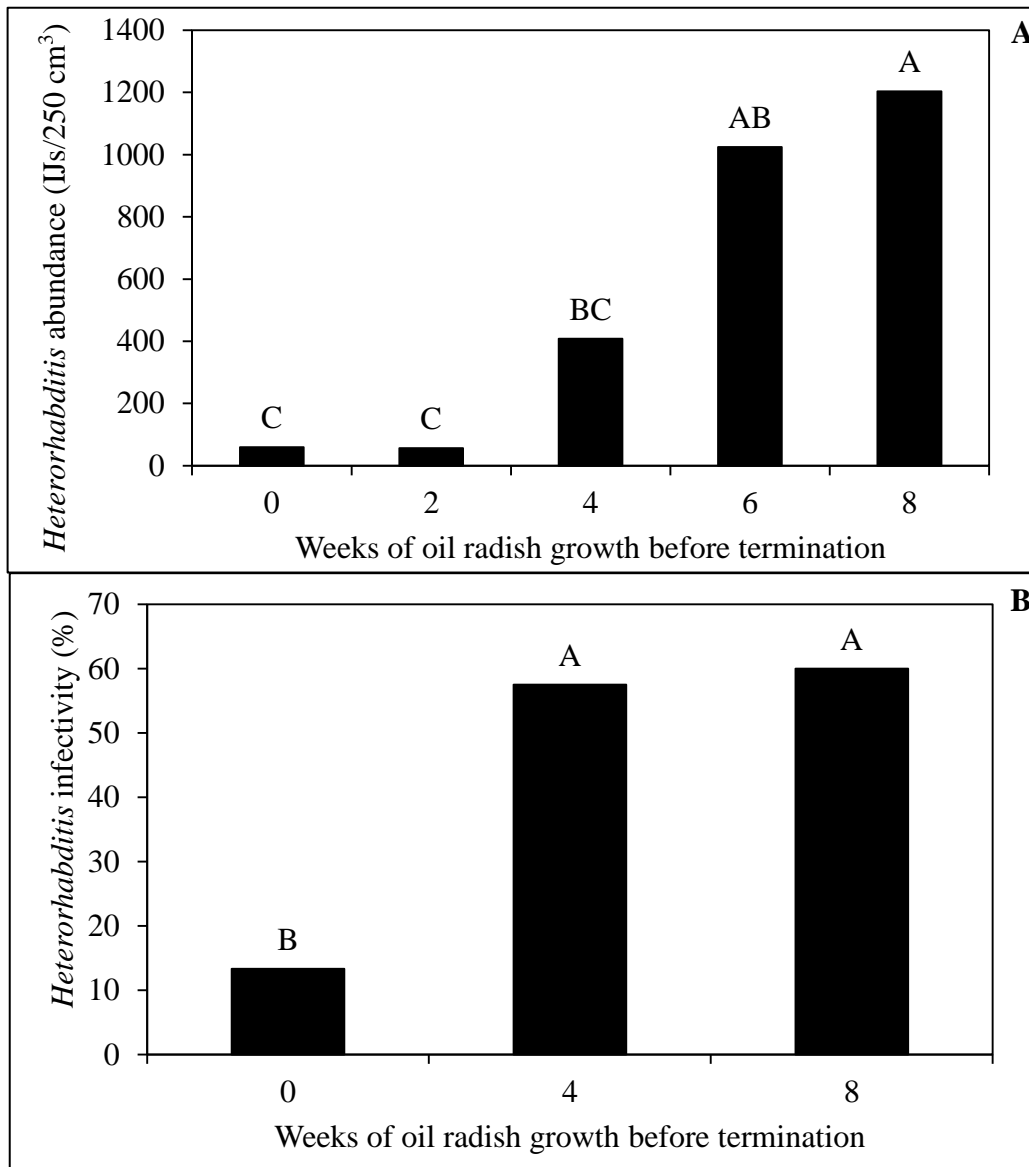


Fig. 3.1. The effect of oil radish age before termination on (A) abundance of *Heterorhabditis* IJs and (B) infectivity of *Heterorhabditis* on mealworms from laboratory larvae bait assay. Means are abundance of *Heterorhabditis* infective juveniles from repeated measures (n = 16) of monthly soil samples taken throughout the growth of pumpkin (*Cucurbita moschata*) planted subsequent to oil radish rate. Means of *Heterorhabditis* infections are from first soil sample after oil radish termination (n = 11), in which one BG replication was removed due to an outlier. Columns in the graph followed by the same letter(s) are not different according to Waller-Duncan k -ratio ($k = 100$) t -test.

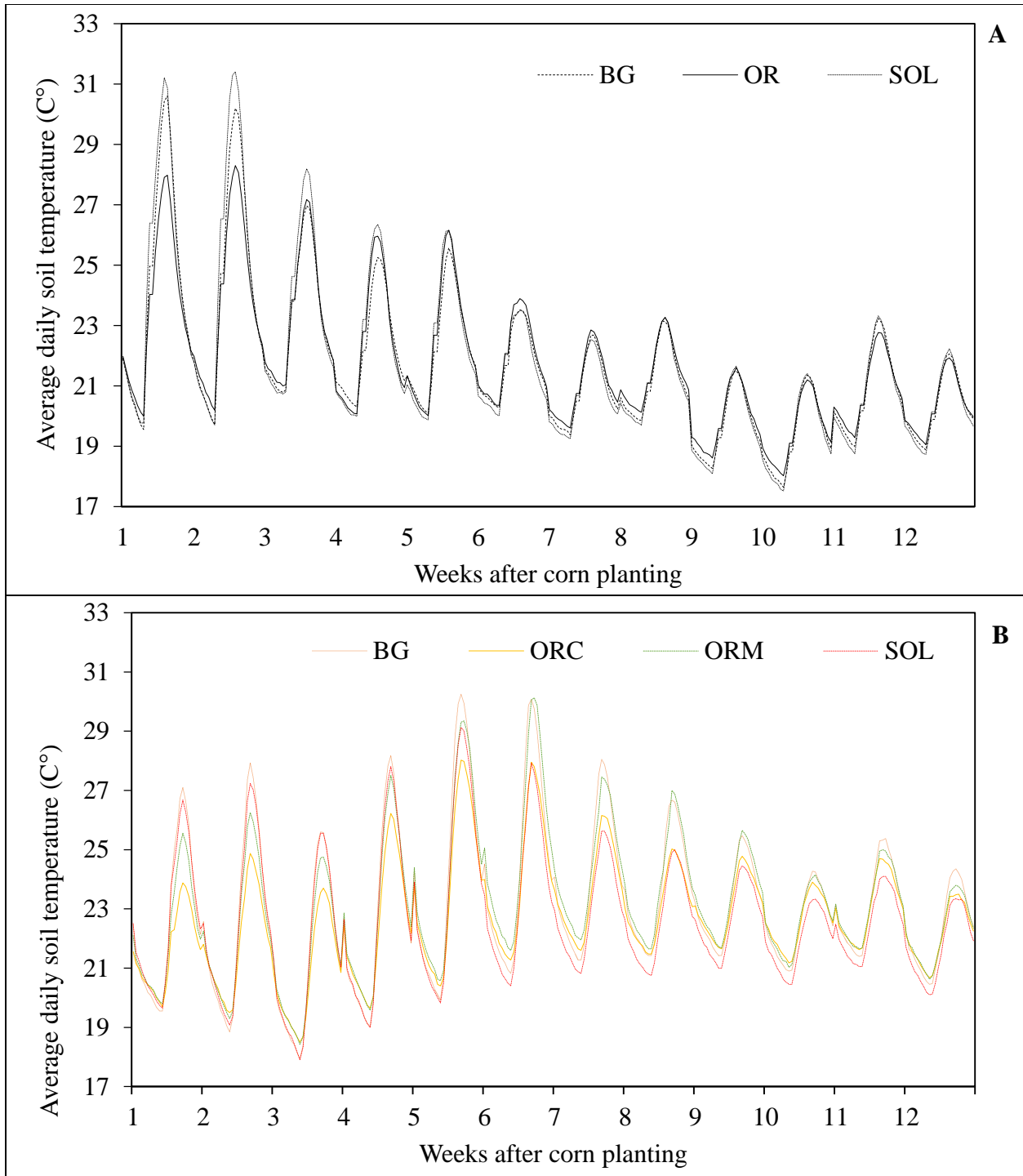


Fig. 3.2. Effects of the pre-plant treatment of oil radish plant residue on no-till (OR, ORC, and ORM) compared to bare ground by conventional tillage (BG) and solarization (SOL) on soil temperature throughout 3 months of corn growing period in A) Trial I and B) Trial II. Means are average of 7-day soil temperature recorded hourly.

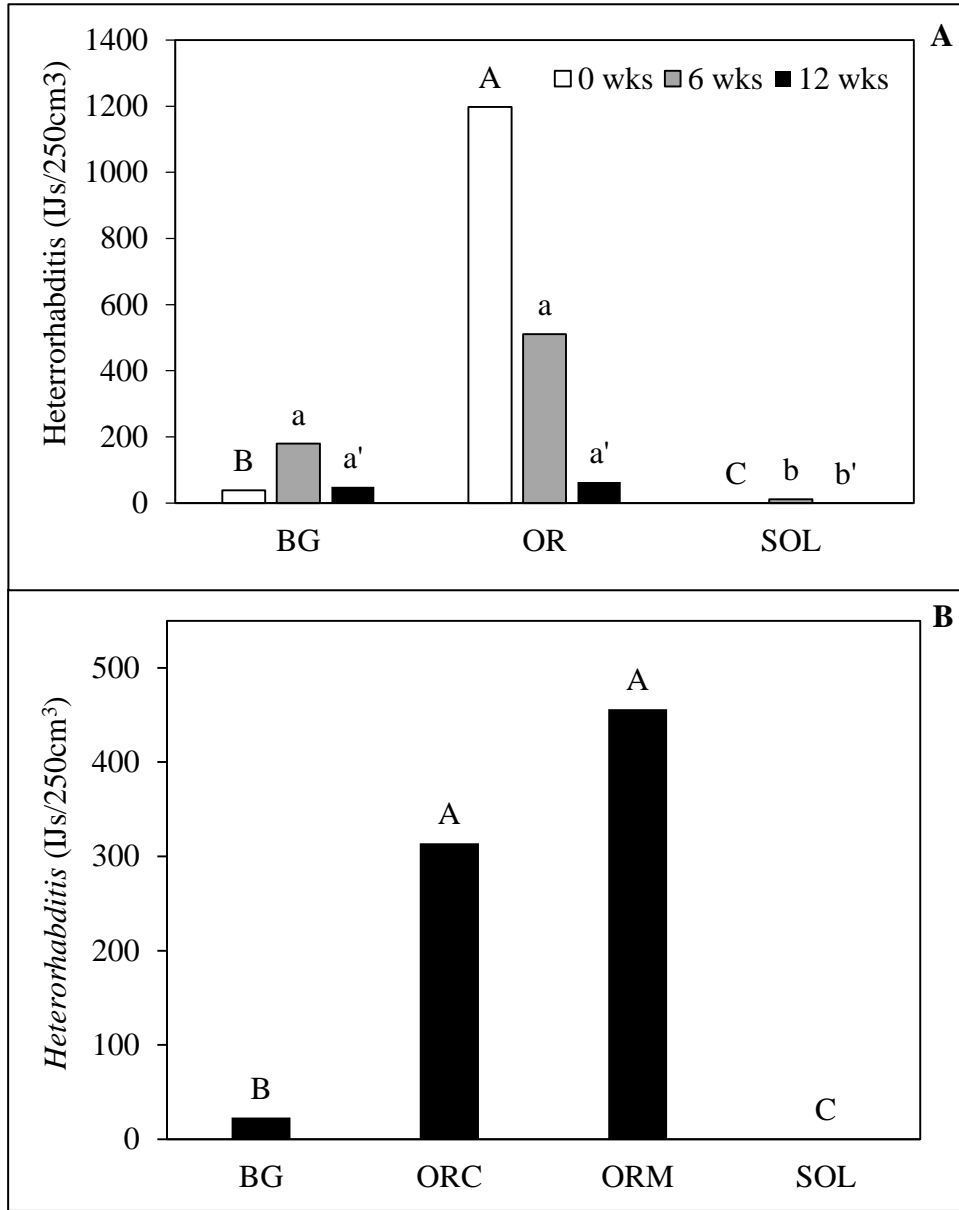


Fig. 3.3. Effect of no-till cover cropping with oil radish (OR, ORC, and ORM) compared to leaving a field bare by conventional tillage (BG), and solarization (SOL) on abundance of *Heterorhabditis* IJs. Means are abundance of *Heterorhabditis* infective juveniles (IJs) from A) Trial I at 3 sampling dates taken monthly at 0, 6 and 12 weeks of corn growth (n = 4), and B) repeated measure of 3 sampling dates of Trial II (n = 36). Columns followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k* = 100) *t*-test.

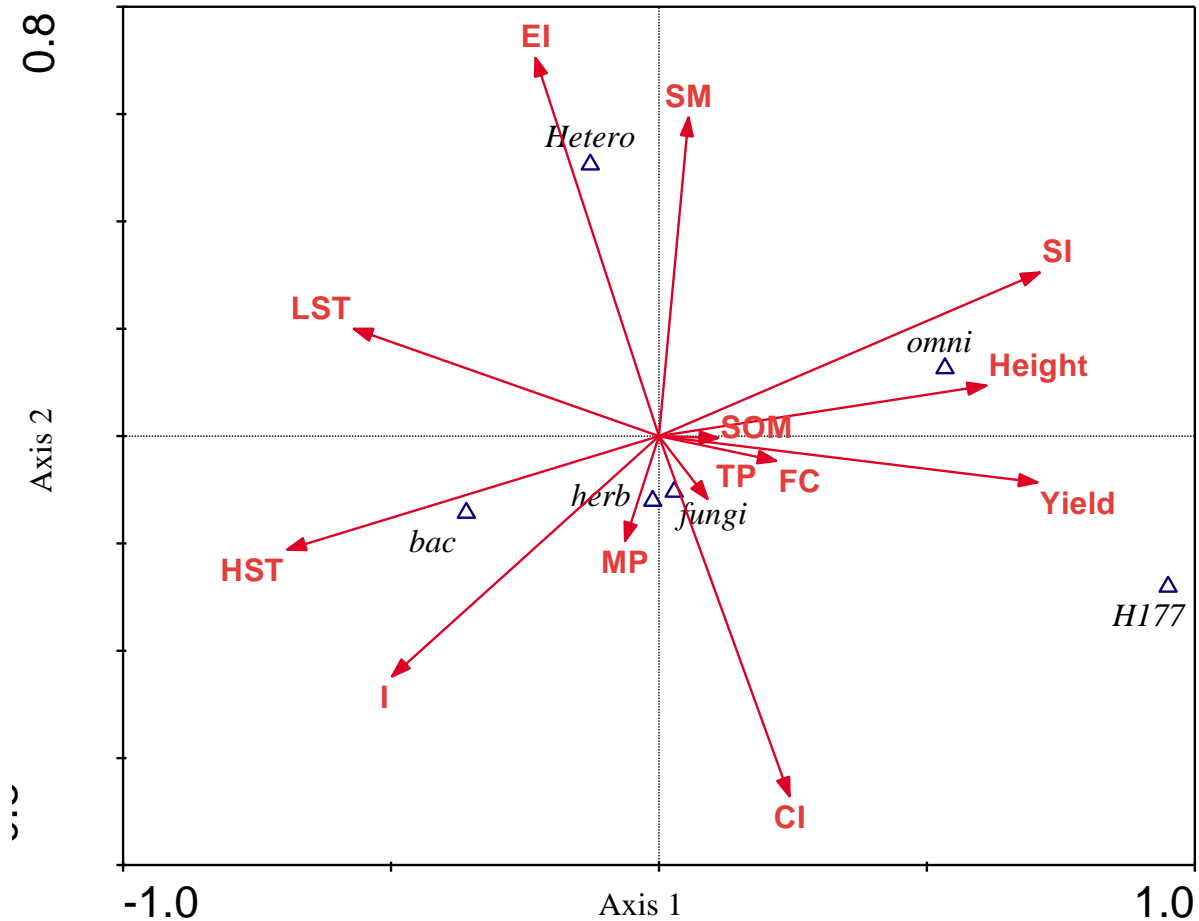


Fig. 3.4. Canonical correspondence analysis of the relationship of free-living nematodes, nematode indices, and soil edaphic factors from all treatments and sampling dates in Trial I. Ordinance diagram depicts the correlation with the first two canonical axes are indicated by the direction of arrows while the length represents the strength of correlations in Trial I. Cumulative percent variance of species-environment relation is 81.8% for both axes. Species variables included in the analysis are nematode food web guilds of bacterivores (bac), fungivores (fungi), herbivores (herb), and omnivores (omni); abundance of *Heterorhabditis* (Hetero); and occurrence of *Heterorhabditis* field cage larvae bait assay of 177 (H177) μm metal sieve. Environment variables included in the analysis are nematode community indices of channel index (CI), enrichment index (EI), and structure index; soil parameters of field capacity (FC), infiltration (I), total porosity (TP), macroporosity (MP), soil organic matter (SOM), soil moisture (SM), lowest daily soil temperature (LST), and highest daily soil temperature (HST); and plant growth parameters of chlorophyll (Chl), height, and yield.

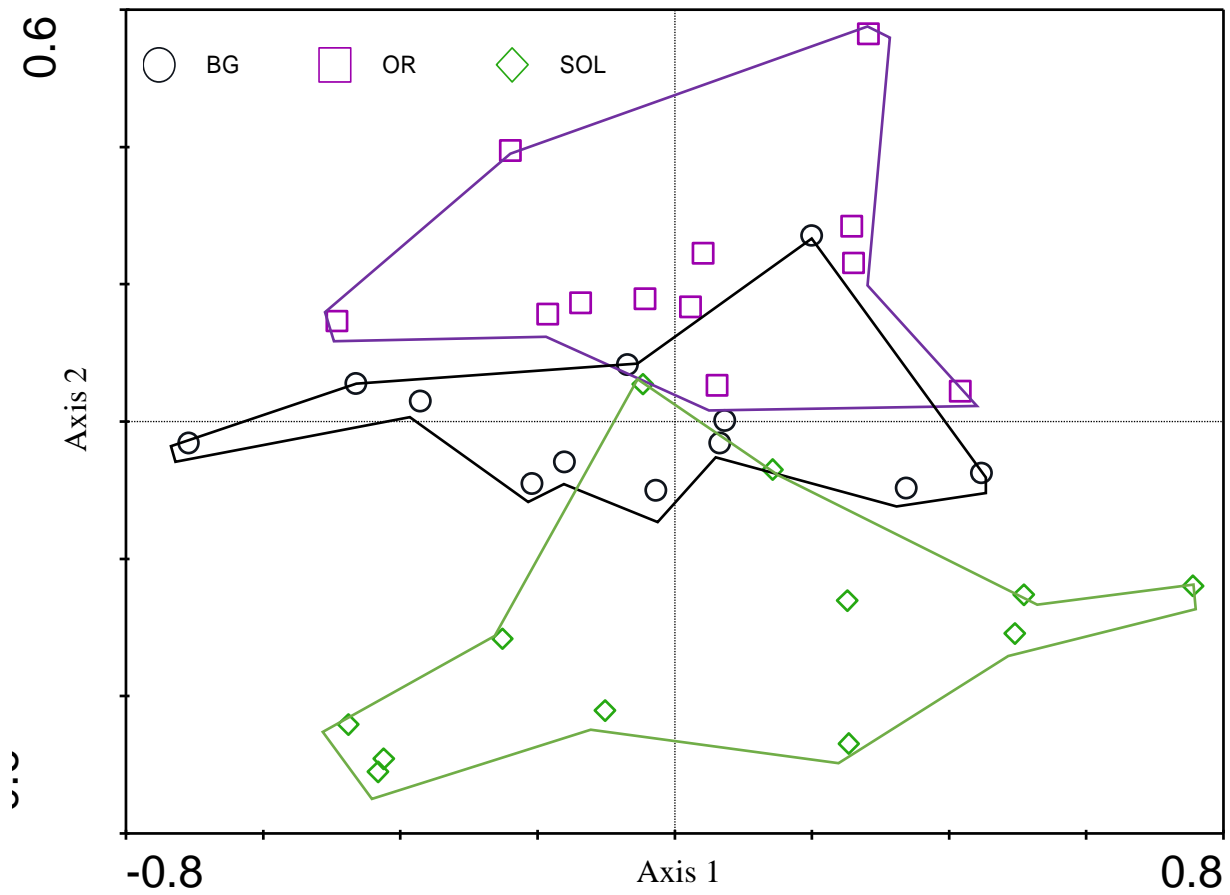


Fig. 3.5. Effects of no-till cover cropping with oil radish (OR) compared to bare ground by conventional tillage practices (BG) and solarization (SOL) on sample distribution on the canonical axes of a canonical correspondence analysis of Trial I. A scatter plot represent to position of samples from each treatment on the first two canonical axes. Boundary line for sample values are color coordinated with treatment color.

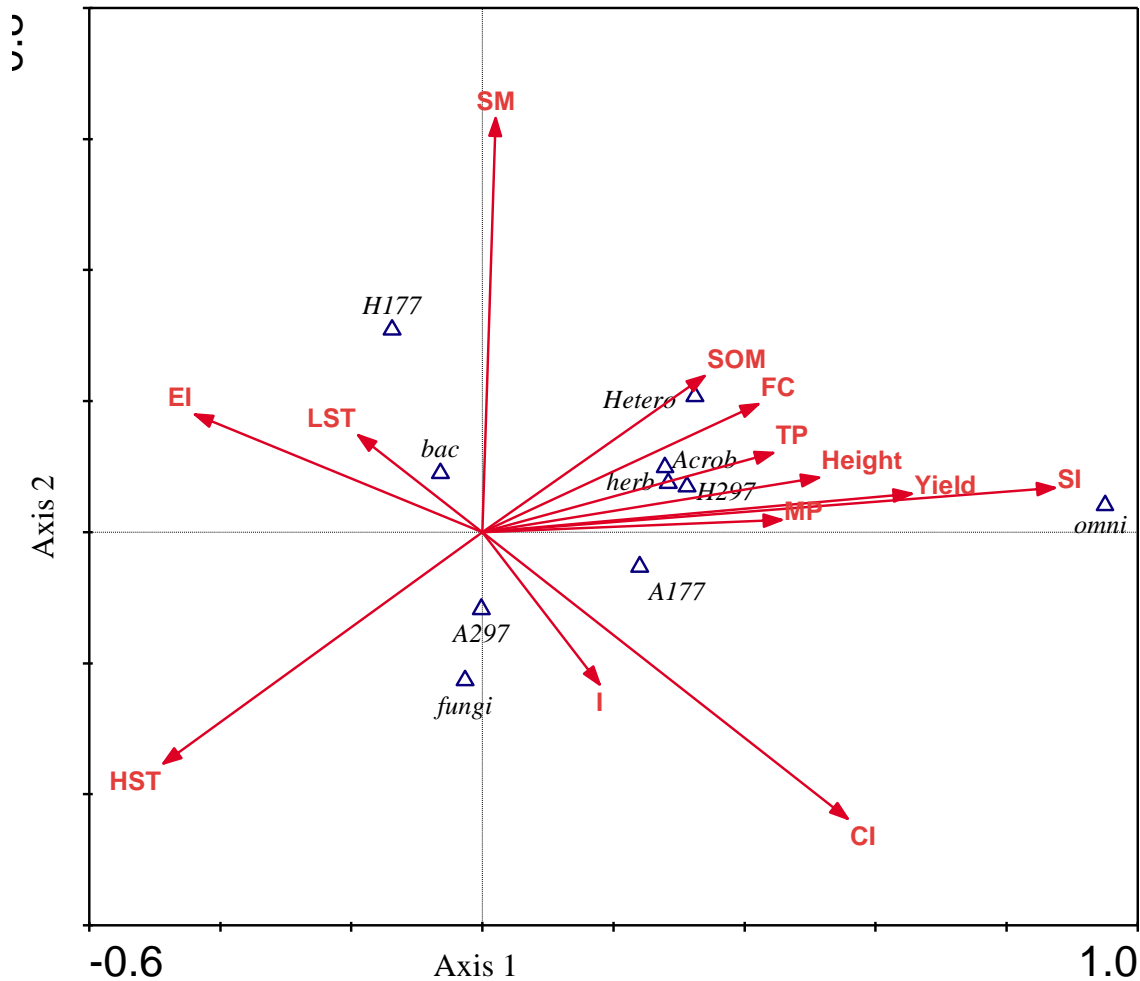


Fig. 3.6. Canonical correspondence analysis of the relationship of free-living nematodes, nematode indices, and soil edaphic factors from all treatments and sampling dates in Trial II. Ordinance diagram depicts the correlation with the first two canonical axes are indicated by the direction of arrows while the length represents the strength of correlations in Trial II. Cumulative percent variance of species-environment relation is 95.0% for both axes. Species variables included in the analysis are nematode food web guilds of bacterivores (bac); fungivores (fungi); herbivores (herb); omnivores (omni); abundance of *Heterorhabditis* (Hetero) and *Acrobeloides* (Acrob); occurrence of *Heterorhabditis* field cage larvae bait assay of 297 (H297) and 177 (H177) μm metal sieve; occurrence of *Acrobeloides* field cage larvae bait assay of 297 (A297) and 177 (A177) μm metal sieve; and thrips (*F. occidentalis*) count from stick traps. Environment variables included in the analysis are nematode community indices of channel index (CI), enrichment index (EI), and structure index; soil parameters of field capacity (FC), infiltration (I), total porosity (TP), macroporosity (MP), soil organic matter (SOM soil moisture (SM), lowest daily soil temperature (LST), and highest daily soil temperature (HST); and plant growth parameters of chlorophyll (Chl), height, and yield.

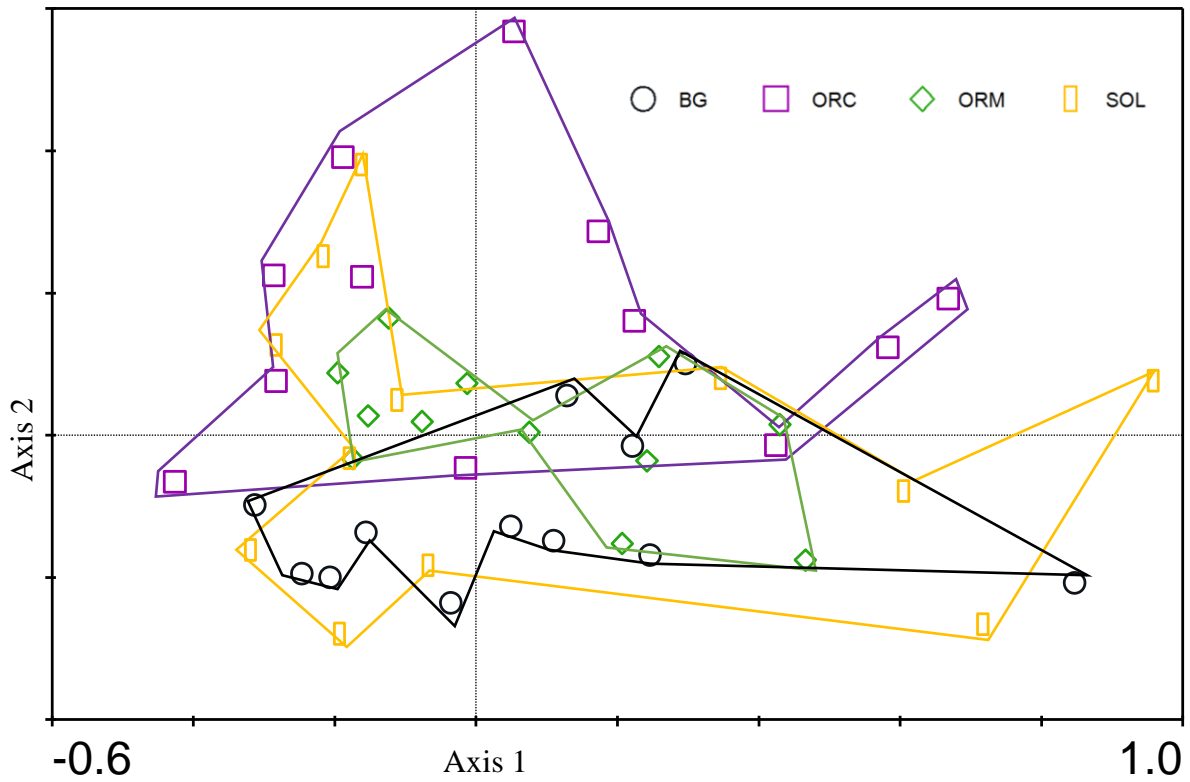


Fig. 3.7. Effects of no-till cover cropping with oil radish terminated by cutting by hand (ORC) and line trimming (ORM) compared to bare ground by conventional tillage practices (BG) and solarization (SOL) on sample distribution on the canonical axes of a canonical correspondence analysis of Trial II. A scatter plot (B) represent to position of samples from each treatment on the first two canonical axes. Boundary line for sample values are color coordinated with treatment color.